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Life Stage and Population Variation in Resistance and Tolerance of *Hyalella azteca* (Amphipoda) to Low pH

R. L. France and P. M. Stokes

Institute for Environmental Studies and Department of Botany, University of Toronto, Toronto, Ont. M5S 1A4

France, R. L., and P. M. Stokes. 1987. Life stage and population variation in resistance and tolerance of *Hyalella azteca* (Amphipoda) to low pH. Can. J. Fish. Aquat. Sci. 44: 1102–1111.

Lethality experiments revealed that resistance of *Hyalella azteca* (Amphipoda) to low pH was directly related to size and developmental stage. Exposure of adults to water below pH 5.0 during pulses of acid snowmelt, or of juveniles to below pH 5.5 through gradual lake acidification, could result in population decline. *Hyalella azteca* from moderately acidic Ontario lakes (pH 5.6–5.7) survived longer at lethal pH than did amphipods from circumneutral lakes (pH 6.4–7.2) where spring pH depressions do not occur. Resistance and tolerance to low pH was neither readily lost by tolerant amphipods exposed to neutral water for 10 d nor readily gained by nontolerant amphipods exposed to sublethal low pH for a similar duration. This absence of physiological plasticity of individual *H. azteca* suggests that population differences in acid tolerance may result from processes of selective mortality with or without a genetic basis.

Des expériences de létalité ont révélé que la résistance de *Hyalella azteca* (Amphipodes) à un faible pH est directement liée à la taille et au stade de développement. L'exposition d'adultes à de l'eau acide à pH inférieur à 5,0 provenant de la fonte des neiges ou de juvéniles à de l'eau à pH inférieur à 5,5 suite à l'acidification lacustre graduelle peut entraîner une baisse des effectifs. *Hyalella azteca* peuplant des lacs ontariens modérément acides (pH 5,6–5,7) ont survécu plus longtemps à un pH létal que des amphipodes provenant de lacs presque neutres (pH 6,4–7,2) où une baisse du pH n'a pas lieu au printemps. La résistance et la tolérance à un faible pH n'étaient ni facilement perdues par des amphipodes tolérants exposés à des eaux neutres pendant 10 j ni aisément acquises par des amphipodes non tolérants exposés à un faible pH sublétal pendant la même période. Cette absence de plasticité physiologique chez les individus de *H. azteca* porte à croire que les différences de tolérance aux acides peuvent être le résultat d'une mortalité sélective à caractère génétique ou non.

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Tolerance refers to an organism's ability to survive indefinitely when exposed to certain environmental conditions. Resistance implies a temporal effect under a set of stressful conditions which will eventually prove lethal outside the range of tolerance (Fry 1947; Duncan and Klaverkamp 1983). Due to the episodic nature of acid snowmelt and severe storm events on the Canadian Shield (Jeffries et al. 1979; Keller 1983; LaZerte 1984), survival of any species here will depend not only on its tolerance to the magnitude of pH depression, but also on its resistance to the duration of perturbation.

Although short-term laboratory experiments and survey data indicate that amphipods are sensitive to pH below 5.5–6.0 (Borgstrom and Hendrey 1976; Roff and Kwaitkowski 1977; Brehm and Meijering 1982; Meinel et al. 1985; Sutcliffe and Carrick 1973a; Okland and Okland 1985; Stephenson and Mackie 1986), tolerance and resistance may vary over the life cycle and among animals from different populations depending upon their history of exposure.

The objectives of this study were to (1) determine how the tolerance and resistance of *Hyalella azteca* (Amphipoda) to low pH varies in relation to organism size and instar stage, and to (2) ascertain interpopulation variability in low pH resistance of *H. azteca* collected from five lakes of different acidity (pH 5.7–7.2). If population variation was demonstrated, we then planned to investigate whether these differences in resistance were due to the ionic composition of testing media or to processes of short-term acclimation or acclimatization. In this paper, acclimation refers to the physiological alteration of an organism's homeostasis in a compensatory response to experimental manipulation of a few environmental variables; acclimatization occurs through exposure for much longer periods to modifications of the organism's natural habitat (Duncan and Klaverkamp 1983). Decisions regarding the operation of these two processes are based on comparisons of survival rates (e.g. Swarts et al. 1978). Where reference is made to low pH or acid-induced resistance or tolerance, it is understood that the toxic agent that the amphipods are responding to is the hydrogen ion (Brehm and Meijering 1982).

Study Area

Hyalella azteca were collected from five lakes within the Haliburton–Muskoka region of south-central Ontario, an area susceptible to, and currently receiving, acidic precipitation (Dillon et al. 1978; Jeffries et al. 1979; Schieder et al. 1979; LaZerte and Dillon 1984). Glen Lake has hard water (Table 1) and a pH that does not decrease much below the average annual value of 7.23. Blue Chalk and Harp lakes are circumneutral with moderate alkalinities (Table 1); the pH over wide-

TABLE 1. Summary of selected chemistry for lakes of origin of *H. azteca* used for experiments in Paint Lake water. Data are from P. Dillon and K. Holtze, MOE, Dorset Research Centre.

Lake	pH	Alk. ($\mu eq \cdot L^{-1}$)	Colour (Hazen units)	Cond. ($\mu S \cdot cm^{-1}$)	Ca ($mg \cdot L^{-1}$)	Al ($\mu g \cdot L^{-1}$)	Mn ($\mu g \cdot L^{-1}$)
Glen	7.23	1 200	13	169.0	25.0	26	59.3
Blue Chalk	6.47	103	9	30.3	2.6	8	36.7
Crosson	5.65	58	29	27.8	2.1	80	39.6
Harp	6.22	101	22	36.1	2.9	39	64.1
Heney	5.74	46	13	25.0	2.0	41	30.0
Paint	6.15	83	26	62.3	4.2	33	80.0

spread areas of the littoral zone never drops to below 6.0 (P. Dillon, Ontario Ministry of the Environment (MOE), Dorset Research Centre, Dorset, Ont., unpubl. data). The more acid lakes, Crosson and Heney, have average annual pHs of 5.65 and 5.74, respectively, which, however, can decrease to below 5.0 during spring snowmelt (P. Dillon, MOE, unpubl. data). The higher colour value for Crosson Lake suggests that it may have always been somewhat acidic. Both Crosson and Heney lakes have experienced biotic disturbances related to acidification (Harvey 1982; Allison and Harvey 1981; Rooke and Mackie 1984). Stephenson and Mackie (1986) found a 2-wk delay in *H. azteca* recruitment in Heney Lake compared with other circumneutral lakes such as Blue Chalk and Harp where springtime pH depressions did not occur; they suggested that this is indication of a stressed *H. azteca* population.

Materials and Methods

Life Stage Response to Low pH

Experiment A

During August 1984, *H. azteca* were collected by dip samples and gentle sieving from the littoral zone of Glen Lake (Table 1). Amphipods were held for 3 d in aerated aquaria containing a thin layer of flocculent organic sediment from Glen Lake that covered most of the aquaria bottoms and served both as substrate and food supply (Hargrave 1970). Water from Paint Lake was used for both this laboratory adjustment period and the following test. The concentrations (milligrams per litre) of selected ions were as follows: Ca^{2+}, 4.2; Na^{+}, 2.5; Mg^{2+}, 1.5; K^{+}, 0.7; Cl^{-}, 4.1; SO_4^{2-}, 9.6; total Mn, 0.08; and total Cu, 0.002 (K. Holtze, MOE, unpubl. data).

Experiments were conducted in 7-L plastic aquaria, each containing two 100-mL plastic beakers with their sides partially removed and covered with Nitex® screening. To limit the possibility of stress-related antagonism, amphipods for each treatment were separated on the basis of size and contained within the testing beakers. The size range of the larger amphipods was 0.58–0.82 mm head length (age 1+ yr adults) and for the smaller amphipods was 0.32–0.60 mm head length (age 1+ mo juveniles).

Water from Paint Lake was acidified to the test pH with H_2SO_4 and vigorously aerated to ensure removal of excess CO_2 (France 1982). A natural photoperiod and continuous aeration was used for the test. Mortality and pH were checked four to six times daily. At each check the plastic beakers containing the amphipods were raised until most of the water was drained free and were then resubmerged enabling water renewal. Periodic additions of acid were required to maintain each treatment pH. Measured pH values never fluctuated more than ±0.20 unit from the nominal levels, and SES were within 0.01–0.03 pH unit of the mean for all treatments. Dead animals were removed, preserved in 90% ethanol, and measured. Further details of this experiment and any deviations in protocol for subsequent tests are summarized in Table 2.

Experiment B

To confirm that the mortality observed in Experiment A was size dependent, an additional test was undertaken in July 1985 using animals from acidic (pH 5.65) Crosson Lake (Table 2).

Population Variation in Acid Resistance

Experiment C

In late May 1985, an experiment was undertaken to compare the low pH sensitivities of overwintering adult *H. azteca* from three populations that differed in their history of pH exposure (Table 2). Amphipods were collected from Glen Lake (pH 7.23) and from Blue Chalk and Crosson lakes (pH 6.40 and 5.34 at time of collections, respectively; cf. Table 1 for mean annual values). Amphipod head lengths from two randomly chosen Blue Chalk treatments were not significantly different from either Crosson or Glen Lake treatments (ANOVA test, $p < 0.05$), indicating that differences in rate of mortality cannot be ascribed to differences in organism size. Amphipod sizes were similarly not significantly different among treatments for the other experiments (Table 2).

Experiment D

In early July 1985, we compared the low pH resistance of adult *H. azteca* from acidic Heney Lake (pH 5.74) and circumneutral Harp Lake (Table 2).

Testing Media Comparison (Influence of Ionic Strength on Acid Resistance)

During early July 1985, we investigated (Table 2) whether the large differences in "low pH" resistance found between adult amphipods from Glen and Crosson lakes could have been due to an effect of ionic stress on Glen Lake animals following their transfer from Glen Lake to the much softer test water of Paint Lake. The following experimental media were used: unmodified Paint Lake water as before (43.4 $\mu S \cdot cm^{-1}$, 4.0 mg $Ca \cdot L^{-1}$); Paint Lake water to which $CaCl_2$ had been added (48.1 $\mu S \cdot cm^{-1}$, 10.0 mg $Ca \cdot L^{-1}$); untreated Glen Lake water (105.23 $\mu S \cdot cm^{-1}$, 23.0 mg $Ca \cdot L^{-1}$); and water from Glen Lake diluted 50% by volume with distilled water (56.7 $\mu S \cdot cm^{-1}$, 13.0 mg $Ca \cdot L^{-1}$).

Influence of Previous Exposure on Acclimation to Low pH

During September 1984, adult *H. azteca* were collected

TABLE 2. Details of experimental methodology.

	Life stage		Population variation				
	Exp. A	Exp. B	Exp. C	Exp. D	Testing media	Acclimation induction	Acclimatization loss
Source of animals	Glen	Crosson	Blue Chalk Glen Crosson	Heney Harp	Glen Crosson	Glen	Blue Chalk Crosson
Adjustment period (d)	3	2	1	2[a]	2[a]	10[b]	10
Treatment pHs	3.5, 4.0, 4.2, 4.5, 4.8, 5.0, 5.5, 6.4	4.0, 4.3, 4.6	4.1, 4.5, 4.8, 5.2, 6.3	4.0, 4.3, 4.6, 4.9, 6.2	4.0, 4.3, 4.6[b]	4.0, 4.2, 4.5, 4.8, 5.0	4.0, 4.2, 4.5, 4.8, 5.0, 6.4
Replicates	3	1	3	3	1	1–3[b]	3
Organisms per rep.	12–16	15	11–13	12–17	11–15	10–12	10
Head length (mm ± SE)	0.32±0.60 0.58±0.82	0.31±0.68	0.67±0.03 0.70±0.04 0.69±0.02	0.68±0.07 0.70±0.03	0.64±0.11 0.70±0.10	0.62±0.11 0.68±0.08	0.48±0.08 0.51±0.08
Food source	Tetra-min, floc	Floc	Floc	Floc	None	Tetra-min, floc	None
Temperature (°C)	19–25	15–22	12–21	17–24	16–24	12–20	15–24
Water renewal	days 3 and 6	None	day 3	None	None	day 3	None
Exptal duration (d)	8	8	8	8	8	8	10

[a]In water of lake of origin.
[b]Described in text.

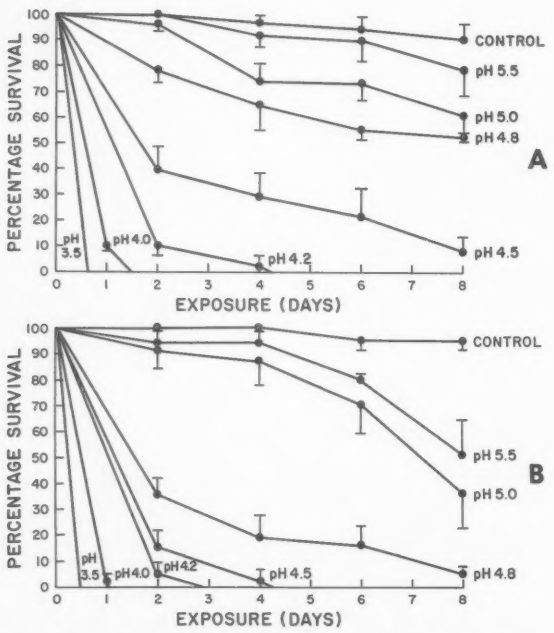

FIG. 1. Survival ± SE (n = 3) of (A) age 1+ yr adult (0.58–0.82 mm head length) and (B) age 1+ mo juvenile (0.32- to 0.60-mm head length) *H. azteca* in depressed pH. Control pH was 6.4. Source of amphipods was Glen Lake (pH 7.23).

from Glen Lake and held for 10 d in Paint Lake water adjusted to mean pH 6.56 ± 0.03 (SE) (n = 24), 5.86 ± 0.03 (39), 5.56 ± 0.02 (41), and 5.19 ± 0.03 (41). These are nominally represented as pH 6.6 (control), 5.9, 5.6, and 5.2. The following experimental design was used (Table 2): amphipods from the control and pH 5.9 preexposures were tested at lethal pHs 5.0 (two replicates), 4.8, 4.5, and 4.2 (three replicates at each pH), and pH 4.0 (one replicate); amphipods from pH 5.6 preexposure were tested at pH 4.8, 4.5, and 4.2 (three replicates at each pH); and amphipods from pH 5.2 preexposure were tested at pH 4.8, 4.5, and 4.2 (one replicate).

Influence of Exposure to Neutral pH on Loss of Acclimatization

In late August 1985, we examined whether amphipods from an acidic lake would lose their resistance to low pH through short-term exposure to neutral conditions. Late juvenile – early adult amphipods were collected from Blue Chalk (pH 6.47) and Crosson (pH 5.65) lakes and held in Paint Lake water (pH 6.45) for 10 d prior to testing (Table 2).

Statistical Analysis

Median lethal concentrations (LC50) and survival times (MST) were calculated and compared using the procedures of Litchfield (1949) and Litchfield and Wilcoxon (1949). Because of the potential for misapplication of fiducial limits when testing differences (Hodson et al. 1977), the average MSTs were calculated independently from replicate MSTs in the acclimation experiment. Significant differences between survival curves were determined with the nonparametric Kolmogorov–Smirnov (K-S) test for cumulative distributions (Siegel 1956). Differences in tolerance and resistance to low pH are indicated by differences between LC50 values and between survival times, respectively (Duncan and Klaverkamp 1983).

Results

Life Stage Differences in Acid Resistance and Tolerance (Experiments A and B)

Acidification to pH < 5.0 for adults and to pH < 5.5 for juvenile *H. azteca* caused increased mortality (Fig. 1). Acute toxicity occurred below pH 4.2 for adults and below pH 4.5 for juveniles. By 8 d, mortality had stabilized for the adults but not for the juveniles.

With the exception of pH 5.0, the MSTs between adults and juveniles were different ($p < 0.05$) for all treatments (Table 3).

TABLE 3. Median survival times (days) ± 95% fiducial limits and slope functions (S) of amphipods after exposure for 8 d to low pH. RR = reaction time ratio; SR = slope function ratio; *difference at $p < 0.05$. Control water was pH 6.4.

	Adults		Juveniles			
pH	MST	S	MST	S	RR	SR
Control	Indefinite	—	Indefinite	—	—	—
5.5	23.75 ± 8.54	3.17	8.54 ± 1.71	1.64	2.78*	1.93*
5.0	9.79 ± 2.94	2.73	7.16 ± 1.39	1.56	1.37	1.75*
4.8	8.13 ± 4.74	5.90	1.79 ± 0.66	2.32	4.54*	2.54*
4.5	1.88 ± 0.90	2.99	0.79 ± 0.13	1.56	3.62*	1.92*
4.2	1.18 ± 0.28	1.71	0.52 ± 0.10	1.52	2.27*	1.13
4.0	0.56 ± 0.12	1.71	0.25 ± 0.03	1.32	2.40*	1.30*
3.5	0.32 ± 0.09	2.02	0.17 ± 0.04	1.66	1.88*	1.21

TABLE 4. Median survival times (days) ± 95% fiducial limits and slope functions (S) of *H. azteca* collected from five lakes after exposure to low pH for 8 d. Control water was pH 6.2.

	Crosson		Blue Chalk		Glen			Heney		Harp	
pH	MST	S	MST	S	MST	S	pH	MST	S	MST	S
Control	Indefinite	—	Indefinite	—	Indefinite	—	Control	Indefinite	—	Indefinite	—
5.2	Indefinite (?)	—	83.33 ± 10.32	4.90	9.17 ± 1.95	1.83	4.9	Indefinite (?)	—	66.80 ± 21.57	3.81
4.8	Indefinite (?)	—	26.67 ± 4.98	3.27	6.54 ± 1.04	1.57	4.6	Indefinite (?)	—	28.30 ± 10.63	3.78
4.5	166.70 ± 36.30	8.35	13.33 ± 2.67	1.77	2.71 ± 0.59	1.91	4.3	15.00 ± 3.97	2.76	7.08 ± 2.74	4.25
4.1	6.92 ± 0.85	1.38	3.25 ± 0.69	1.86	1.29 ± 0.19	1.47	4.0	9.17 ± 2.37	2.75	2.50 ± 0.70	2.17

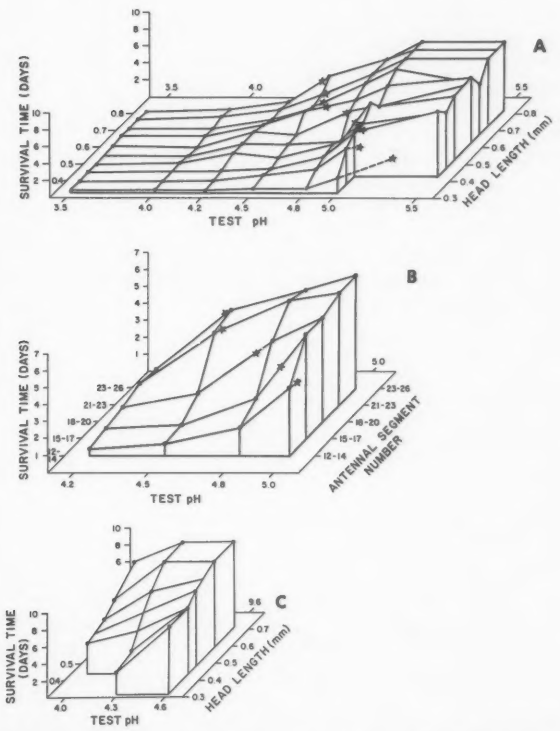

FIG. 2. Relationship of mean survival time of Glen Lake amphipods to (A) head length and (B) antennal segment number and of Crosson Lake amphipods to (C) head length. *Median tolerance limit (LC50) of particular size or age class.

Slope functions also differed ($p < 0.05$) for five of the seven treatments indicating that the time course of mortality was dissimilar. Chi-square tests (Litchfield and Wilcoxon 1949) showed that eye-fitted toxicity lines provided a satisfactory fit to the experimental results. The asymptotic LC50 calculated at 8 d for the adults was pH 4.95 ± 0.16 (S = 1.05), which differed ($p < 0.05$) from the 8-d LC50 of pH 5.30 ± 0.17 (S = 1.06) for juveniles. Compared with 1+ mo old juveniles, 1+ yr old adults were twice as tolerant to low pH.

Differences in low pH resistance of amphipods designated as either "adults" or "juveniles" within the pH range 4.2–5.0 were due to size-dependent mortality (Fig. 2A). Below pH 4.2, death was so rapid as to negate benefits of larger size; above pH 5.0, mortalities were rare and independent of amphipod size. The LC50 ranged from pH 4.80 for 0.80–0.85 mm head length amphipods to pH 5.35 for 0.30–0.35 mm head length amphipods. Resistance to low pH can also be explained by instar developmental stage (Fig. 2B). Here, the LC50 ranged from pH 4.48 for *H. azteca* with 22–26 antennal segments to pH 5.08 for amphipods with 12–14 segments.

A size-dependent relationship to pH mortality was also evident for *H. azteca* from Crosson Lake (Fig. 2C). These amphipods were much more resistant to low pH than similarly sized amphipods from Glen Lake.

Population Differences in Acid Resistance (Experiments C and D)

The nonparametric K-S two-sample test showed that mortality of Glen Lake amphipods occurred more rapidly ($p < 0.05$) than mortality of Blue Chalk or Crosson Lake amphipods at every pH treatment (Fig. 3A, 3B). The survivorship curve of Blue Chalk Lake amphipods was statistically different from that for Crosson Lake amphipods at pH 4.1 but not at other

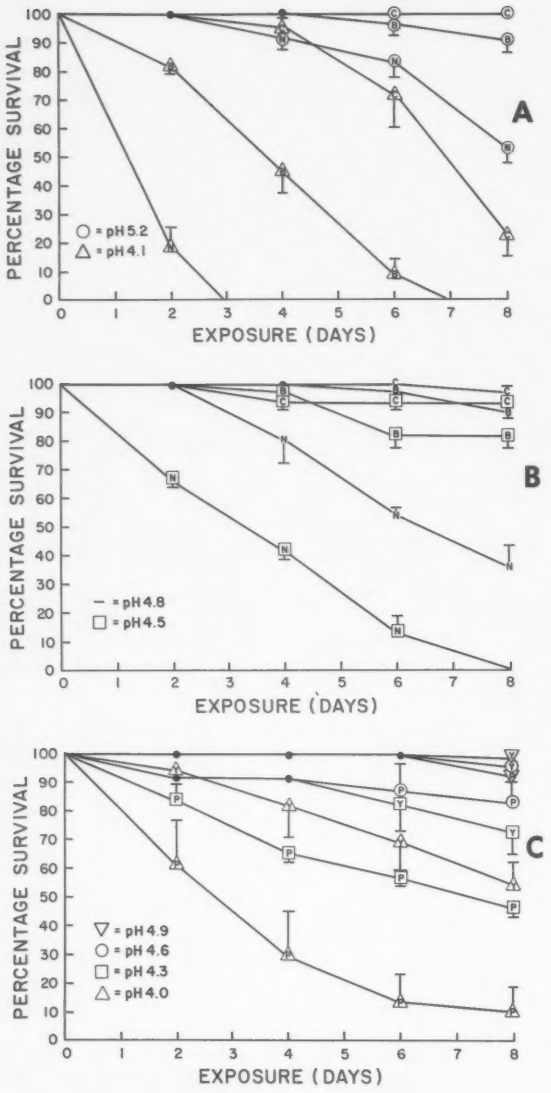

FIG. 3. Comparative survival ± SE (n = 3) of (A and B) *H. azteca* from Crosson (C) (pH 5.65), Blue Chalk (B) (pH 6.47), and Glen (N) (pH 7.23) lakes and of (C) *H. azteca* from Heney (Y) (pH 5.74) and Harp (P) (pH 6.22) lakes, to H^+ toxicity. Control water was pH 6.2–6.6.

pHs. Survival of amphipods from Heney Lake was significantly greater than that of Harp Lake amphipods at pH 4.0 and 4.3, but not at pH 4.6 and 4.9 (Fig. 3C).

Analysis of MSTs (Table 4) confirmed trends evident from survival curve testing. Calculated MSTs among populations were different ($p < 0.05$) at every pH treatment. In general, the range of population resistance was Crosson = Heney > Blue Chalk = Harp > Glen. Therefore, *H. azteca* from moderately acidic lakes which experience spring pulses of acidic snowmelt have greater resistance to lethal low pH than do those from circumneutral lakes.

TABLE 5. Median survival times (days) ± 95% fiducial limits and slope functions (S) of amphipods collected from two lakes, held in pH 6.45 water for 10 d, and then tested at depressed pH for 10 d.

	Crosson		Blue Chalk	
pH	MST	S	MST	S
4.8	116.67 ± 68.00	6.75	9.58 ± 1.46	1.64
4.5	41.67 ± 20.80	5.26	7.50 ± 1.35	1.75
4.2	6.25 ± 1.29	1.92	2.92 ± 0.82	1.93
4.0	5.63 ± 1.01	1.73	2.04 ± 0.34	1.68

Effect of Testing Media on Acid Resistance

Calculated MSTs for amphipods from Crosson Lake were longer ($p < 0.05$) than those derived for Glen Lake amphipods at every pH treatment in all four testing media (Fig. 4). Clearly, differences in low pH resistance between *H. azteca* from these two lakes were not due to an effect of Ca stress on Glen Lake amphipods following their transfer to softer test water.

Effect of Previous Exposure on Acid Resistance and Tolerance

No mortality occurred during the pH 6.6 and 5.9 pretreatments, but approximately 10 and 40% mortality occurred in the pH 5.6 and 5.2 pretreatments, respectively. Survival of *H. azteca* preexposed to pH 5.6 and 5.9 and tested at pH 4.5 was greater than control animals (K-S test, $p < 0.05$), suggesting acclimation (Fig. 5). Mortality of amphipods preexposed to pH 5.6 and 5.2 was more rapid than control animals when tested at pH 4.8, indicating a reverse of acclimation, or sensitization. There were no statistical differences in survival curves for preexposed treatments compared with controls for other combinations.

Analysis of MSTs (Fig. 6) confirmed results from survival curve testing. Of 11 comparisons, seven preexposure MSTs (combined data) were not different ($p < 0.05$) from the controls, while four were lower ($p < 0.05$). Two of these were from preexposure treatments to pH 5.2, indicating sensitization. Of the seven possible parametric comparisons, six preexposure MSTs were not different (t-test, $p > 0.05$) from controls, while preexposure to pH 5.9 significantly reduced the MST at pH 5.0 ($p < 0.10$), again indicative of sensitization and not acclimation of resistance. Preexposure to pH 5.2, 5.6, and 5.9 did not increase tolerance of *H. azteca* tested at lethal pH (Fig. 7). In fact, reduced tolerance was shown for amphipods preexposed to pH 5.2 and 5.6 on days 2 and 4, respectively.

Effect of Exposure to Neutral pH on Acid Resistance and Tolerance

Mortality at all pHs was more rapid for amphipods from Blue Chalk Lake than for those from Crosson Lake ($p < 0.05$) (Fig. 8). Calculated MSTs for *H. azteca* from Crosson Lake remained larger ($p < 0.05$) than those for Blue Chalk amphipods at every test pH despite pretreatment exposure (Table 5). The asymptotic LC50 calculated at 10 d for Crosson Lake amphipods was pH 4.32 ± 0.09 (S = 1.06), which differed ($p < 0.05$) from the 10-d LC50 of pH 4.78 ± 0.14 (S = 1.05) for Blue Chalk amphipods. Further, survival times of adults from Crosson lake were similar to those calculated for this life stage from Experiments B and C. Short-term exposure to

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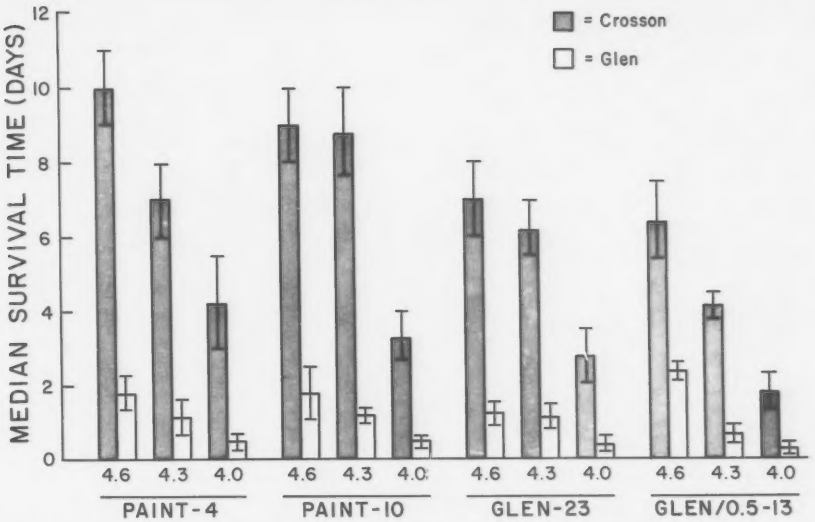

FIG. 4. Median survival times ± 95% fiducial limits of *H. azteca* from Crosson and Glen lakes exposed to pH 4.6, 4.3, and 4.0 in four different testing media. Paint-4 and Paint-10 = Paint Lake water of 4 and 10 mg $Ca \cdot L^{-1}$, respectively; Glen-23 and Glen/0.5–13 = untreated (23 mg $Ca \cdot L^{-1}$) and diluted 13 mg $Ca \cdot L^{-1}$) Glen Lake water, respectively.

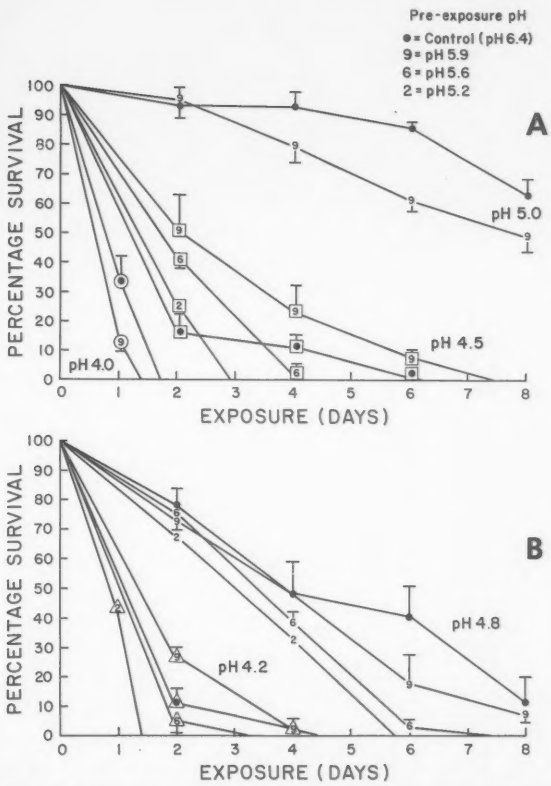

FIG. 5. Survival ± SE of *H. azteca* from Glen Lake in lethal acidity (pH 5.0, 4.8, 4.5, 4.2, and 4.0) following pretest exposure for 10 d to pH 6.4 (control), 5.9, 5.6, or 5.2. Data are graphed separately only for clarity.

circumneutral pH did not alter resistance or tolerance of *H. azteca* from a low pH lake.

Discussion

Low pH Sensitivity

Our tolerance experiments corroborate synoptic data on amphipod sensitivity to low pH. The asymptotic LC50 ranged from pH 4.32 to 4.95 depending on lake of origin. These values are similar to the 4- and 10-d LC50s of pH 4.4 and 4.5, respectively, determined for *H. azteca* from Harp Lake by Stephenson and Mackie (1986). The LC50 for Glen Lake amphipods increased from pH 4.3 on day 1 to pH 4.7 on day 4 and then further to pH 4.9 on day 8. This was the result of protracted mortality. The ultimate pH tolerance level for *H. azteca* is expected to be slightly higher. For example, Zischke et al. (1983) found that *H. azteca* were almost completely eliminated from an experimental channel acidified to pH 5 over a period of several months. The early spring population of *H. azteca* in Canadian lakes consists entirely of adults overwintering from the previous summer (e.g. Hargrave 1970). At pH 4.2–4.6, which can occur during the spring melt of acid snow in Ontario, the MSTs were between 3 and 23 d. This implies that these animals could be vulnerable to spring pH depressions (France and LaZerte 1987).

The susceptibility of *H. azteca* to toxic levels of acidity is strongly dependent on body size and instar stage. *Hyalella azteca* of 0.30–0.35 mm head length are only one third as tolerant as those 0.80–0.85 mm in size. Similarly, *H. azteca* with 12–14 antennal segments, representing amphipods of about instar stage 3 (L. Richman, University of Toronto, Toronto, Ont., unpubl. data), were one fifth as tolerant as 9th instar adults (22–26 antennal segments). It is not known if this age-specific tolerance of *H. azteca* to low pH is due to processes of physiological maturation or simply to changes in

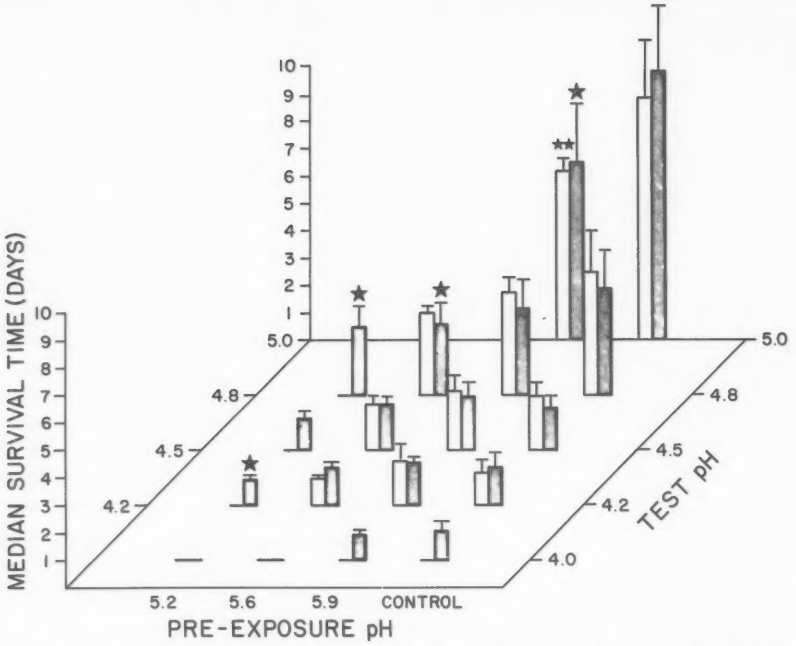

FIG. 6. Median survival times (MST) of amphipods in lethal acidity (test pH 4.0–5.0) following preexposure to pH 5.2, 5.6, 5.9, and 6.4 (control) for 10 d. Open histogram = average MST ± SE from independently calculated replicate MSTs per treatment; hatched histogram = average MST ± 95% fiducial limits from combined data per treatment as in Fig. 5. **Significant difference ($p < 0.10$) from control treatment with parametric t-test; *significant difference ($p < 0.05$) from control treatment with reaction time ratios.

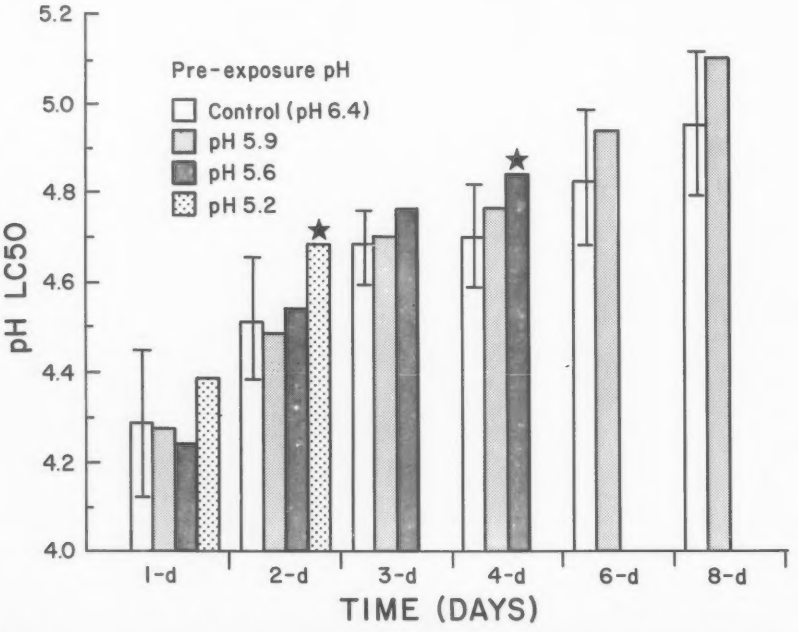

FIG. 7. Low pH LC50 values at six specific times for amphipods exposed to pH 6.4, 5.9, 5.6, or 5.2 for 10 d. Vertical bars represent 95% fiducial limits; *significant difference ($p < 0.05$).

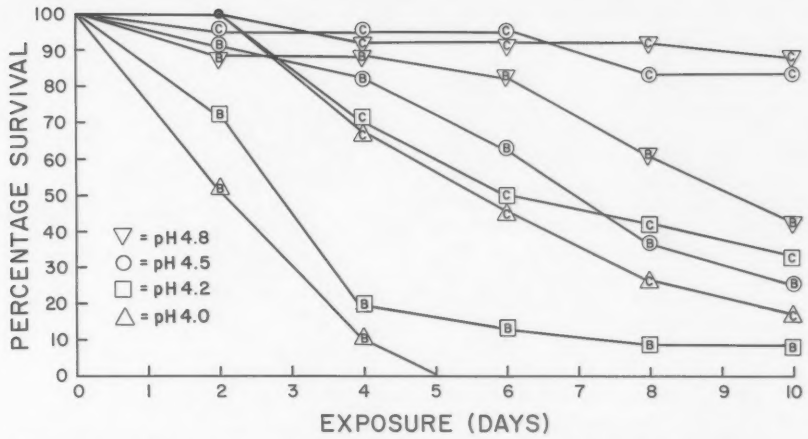

FIG. 8. Comparative survival of *H. azteca* from Crosson (C) and Blue Chalk (B) lakes in lethal acidity after preexposure to control (pH 6.5) water for 10 d. Calculated SEs (not shown) were all within ±25% of the mean.

general body size. In common with other crustaceans, H^+ toxicity in amphipods is due to failures in ionic regulation (Meinel et al. 1985). Small organisms have a greater body and gill surface area per unit weight than do larger animals, allowing for a more rapid loss of ions.

The calculated LC50 of pH 5.35 for 0.30–0.35 mm juvenile *H. azteca* indicates that they are almost as sensitive to low pH as *Mysis relicta* (Nero and Schindler 1983). Nilssen et al. (1984) believed that the marked sensitivity of amphipods to low pH was a result of greater body permeability compared with other benthic macroinvertebrates. Individuals in earlier instar stages are probably even more sensitive, as are postmolt animals due to increases in water uptake and body permeability at this time (Lockwood and Andrews 1969). Our laboratory results support Stephenson and Mackie's (1986) contention that absence of *H. azteca* from lakes below about pH 5.6 could be due to direct H^+ toxicity. Pending detailed life history data on *H. azteca* in Crosson and Heney lakes (e.g. calculation of mortality rates), we suggest that these amphipod populations may be experiencing acidification stress. Support for this hypothesis is based on the greater resistance to lethal low pH for *H. azteca* from the more acid lakes (cf. Luoma 1977; France 1987).

Influence of Testing Media on Survival

Rahel (1983) believed that the greater acid tolerance of yellow perch (*Perca flavescens*) from bog lakes could, in part, be a consequence of physiological adaptation to low electrolyte concentrations in those waters (9–22 $\mu S \cdot cm^{-1}$) compared with the more saline non-bog lakes (49–73 $\mu S \cdot cm^{-1}$). The conductivity range of 25–36 $\mu S \cdot cm^{-1}$ in the softwater lakes from which *H. azteca* were collected is too narrow to account for the differences in population tolerance observed in this study. Comparison of resistance times for *H. azteca* from Glen Lake (169 $\mu S \cdot cm^{-1}$) held in Paint Lake water (62 $\mu S \cdot cm^{-1}$) for either 3 or 10 d before testing (Table 3; Fig. 6) indicated no differences ($p > 0.05$). This agrees with Sutcliffe's (1967) observations that amphipods taken from hardwater localities can quickly and easily readjust their Na balance in response to low environmental concentrations. Population differences in acid tolerance in the present experiments are not due to shifts in chemistry from lake of origin to testing media.

Because failure in ability to restrict rate of Na loss is the primary cause of mortality of amphipods at low pH (Meinel et al. 1985; Sutcliffe and Shaw 1968), it is not surprising that their occurrence in waters near their lower pH tolerance limits is to some extent controlled by the availability of cations (Okland and Okland 1985; Sutcliffe and Carrick 1973b; Schrimpff and Foeckler 1985). Therefore, at low pH, a direct relationship might be expected between the ionic strength of the water and *H. azteca* survival. However, as amphipods have a high Na affinity that is easily saturated at low external concentrations (Sutcliffe 1967), the range in concentrations from 2.5 $mg \cdot L^{-1}$ in untreated Paint Lake water to 17 $mg \cdot L^{-1}$ in Glen lake water is not expected to influence amphipod survival (cf. Meinel et al. 1985). Further, amphipod survival at low pH appears independent of variation in external Ca concentration (Meinel et al. 1985; R. France and P. Stokes, unpubl. data).

Population Variation in Acid Tolerance

The difficulty in comparing relative sensitivities among animals that differ in their history of acid exposure is in identification of underlying mechanisms of induced tolerance. Such differences in population tolerance could result from (1) acclimatization of individuals over their life span to high toxicant concentrations by preliminary exposure to sublethal concentrations (Guthie, cited in Flick et al. 1982), (2) selective mortality of less tolerant animals leaving a more resistant subpopulation in which tolerance is not necessarily a strongly heritable trait (Swarts et al. 1978; Tome and Pough 1982; Scholfield, cited in Flick et al. 1982), and (3) actual evolutionary genetic adaptation over many generations (Rahel 1983; Edwards and Gjedrem 1979).

For acclimation to occur (Flick et al. 1982), it is essential that both the magnitude and duration of change in water quality be sufficient to stimulate physiological adjustment without exceeding the survival ability of the organisms. Resistance and tolerance of *H. azteca* to lethal acidity were not increased after 10 d of preexposure to sublethal pHs. Frequently, survival

remained unaltered; occasionally an acclimation-induced reduction in survival, or sensitization, was observed. These results confirm other investigations which have failed to rigorously demonstrate acclimation of fish to low pH (reviewed by Spry et al. 1981). However, Swarts et al. (1978) cautioned that early attempts may have failed because acclimation pH levels were either too high or too low relative to the test pH levels, which in turn may have been unrealistically low. Our choice of three pretest acidities, exposing *H. azteca* to sublethal pH 5.9, intermediate pH 5.6, and toxic pH 5.2 (with testing of survivors), followed by testing at five concentrations within the pH range 4.0–5.0, was deemed adequate to dispel several of the criticisms raised against earlier studies. It is possible, however, that the exposure period of 10 d may have been too brief to allow development of physiological compensation. There have been no comparable attempts to induce low pH acclimation in other crustaceans.

Loss of tolerance on return of organisms to control water is consistent with a physiological mechanism induced by a specific stress, the removal of which makes the original adaptation redundant, and whose retention would be an energetic detriment (Dixon and Sprague 1981). In the present case, exposure for 10 d to control conditions, in contrast, did not eliminate the differences in acid resistance and tolerance of *H. azteca* from acidic Crosson Lake compared with circumneutral Blue Chalk Lake.

The failure to demonstrate physiological plasticity of individual *H. azteca* to low pH infers that differences in sensitivity may result from selective mortality with the implied possibility of genetic adaptation. For example, Rahel (1983) found that neither acclimation of yellow perch from alkaline lakes (pH 7.6–7.8) to sublethal low pH nor transfer of yellow perch from acid lakes (pH 4.5–4.6) to control water removed differences in acid tolerance, suggesting to him a genetic basis for the greater tolerance of fish from the acid lakes. The possible mechanism of genetic divergence in adjacent amphipod populations, the adaptive significance of genetic variability to other environmental stresses, and the implications of these theoretical considerations for the success of *H. azteca* populations in lakes undergoing chemical recovery are discussed in France (1987).

Whatever the precise mechanism for tolerance induction, the importance of these results for design of future experiments is crucial (cf. France 1987). As with behavioral studies (France 1985), caution must always be applied in assessing biological responses to low pH, as these can be expected to be markedly different in relation to the exposure history of the organisms under investigation (e.g. Price and Swift 1985). Researchers should therefore attempt to avoid the use of test organisms raised in high pH environments.

Acknowledgements

This work would not have been possible without the generous assistance of K. Holtze in making available the facilities of the MOE toxicology laboratory. P. Dillon is thanked for encouraging us to conduct this research at the Dorset Research Centre and for providing background limnological data. The senior author benefited from numerous insightful conversations with MOE personnel, particularly K. Holtze and N. Hutchinson. S. David patiently measured the amphipod samples. Translations were kindly obtained from the Freshwater Institute through D. Rosenberg.

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Empirical Hypothesis to Explain the Restricted Distribution of *Hyalella azteca* (Amphipoda) in Anthropogenically Acidified Lakes

Robert L. France

Institute for Environmental Studies and Department of Botany, University of Toronto, Toronto, Ont. M5S 1A4

and Bruce D. LaZerte

Ontario Ministry of the Environment, Dorset Research Centre, P.O. Box 39, Dorset, Ont. P0A 1E0

France, R. L., and B. D. LaZerte. 1987. Empirical hypothesis to explain the restricted distribution of *Hyalella azteca* (Amphipoda) in anthropogenically acidified lakes. Can. J. Fish. Aquat. Sci. 44: 1112–1121.

A simple and speculative model is proposed to explain the restricted distribution of *Hyalella azteca* (Amphipoda) within acidified Ontario lakes. The model integrates field chemical and ecological data with results from laboratory toxicological experiments. Frequent spring depressions of pH to below 5.0, measured in the littoral zone of Plastic Lake during 1982, were predicted to cause a mortality of 35.2% to a theoretically constructed amphipod population. A time trend simulation suggested that population decline of *H. azteca* in similar anthropogenically acidified lakes could be caused by such direct mortality due to short-term pH fluctuations. Assumptions and factors not considered by the model which could exacerbate or mitigate the effects of acidic snowmelt to *H. azteca* are discussed.

Les auteurs présentent un modèle simple et théorique pour expliquer la répartition restreinte de *Hyalella azteca* (Amphipodes) dans des lacs ontariens acidifiés. Le modèle combine des données chimiques et écologiques recueillies sur le terrain avec les résultats d'expériences toxicologiques menées en laboratoire. On a ainsi prédit que des chutes printanières fréquentes du pH à un niveau inférieur à 5,0, mesuré dans la zone littorale du lac Plastic en 1982, entraineraient la mortalité de 35,2 % d'une population théorique d'amphipodes. Une simulation de la tendance temporelle porte à croire qu'un déclin de la population de *H. azteca* dans des lacs artificiellement acidifiés peut être le résultat de telles mortalités directement entraînées par des variations à court terme du pH. Les auteurs examinent aussi les hypothèses et les facteurs dont le modèle ne tient pas compte et qui peuvent aggraver ou atténuer l'incidence des eaux acides provenant de la fonte des neiges sur *H. azteca*.

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Environmental managers must be able to predict and evaluate effects of chemical impacts rather than act on damage that is already present; extrapolative methods are therefore needed (NRC 1981: Rosenberg and Resh et al. 1981). Mathematical models have been used to predict the rate of long-term lake acidification (Henriksen 1980), as well as short-term hydrochemical dynamics of springmelt in both streams (Christophersen et al. 1984; Seip et al. 1985) and lakes (Booty and Kramer 1984; Goldstein et al. 1984). The need for procedures and approaches linking laboratory-derived concentration–effect relationships on the *individual* with a measure of ecologically significant effects in the field on the *population* can also be met with the development of predictive mathematical models (NRC 1981; Rosenberg and Resh et al. 1981). These reviews conclude that in some cases the best use of data from short-term toxicity tests is in the proposition of long-term effects via simulation models (e.g. Sadler 1983; Van Winkle et al. 1986).

Hyalella azteca (Saussure) (Amphipoda) is a shallow-water, epibenthic amphipod inhabiting most permanent bodies of water on the Canadian Shield whose monthly mean summer temperatures exceed 10°C (Bousfield 1958). However, survey data (Stephenson and Mackie 1986) indicate that this normally ubiquitous amphipod is absent in Ontario lakes with pHs below 5.6 (alkalinity ≤ 0.0 meq·L^{-1}). These authors suggested that the elimination of *H. azteca* from anthropogenically acidified lakes may be due either to acute toxicity during spring periods when pH is depressed or to recruitment failure through long-term exposure to sublethal levels of hydrogen ions.

The failure of many test methods to take into account the time–concentration interaction makes it difficult to apply laboratory results to field situations where episodes of extreme toxicant concentration are usually of finite duration and can be expected to undergo dramatic fluctuations (Mancini 1983). The hypothesis we wished to investigate was whether the rarity of *H. azteca* in acidified lakes could be explained through mortality directly attributable to pulses of acid snowmelt. This objective was investigated with the development of a very simple and speculative model integrating chemical data from Plastic Lake, an anthropogenically acidified lake within the Haliburton–Muskoka region of south-central Ontario (LaZerte and Dillon 1984), with results from laboratory toxicological

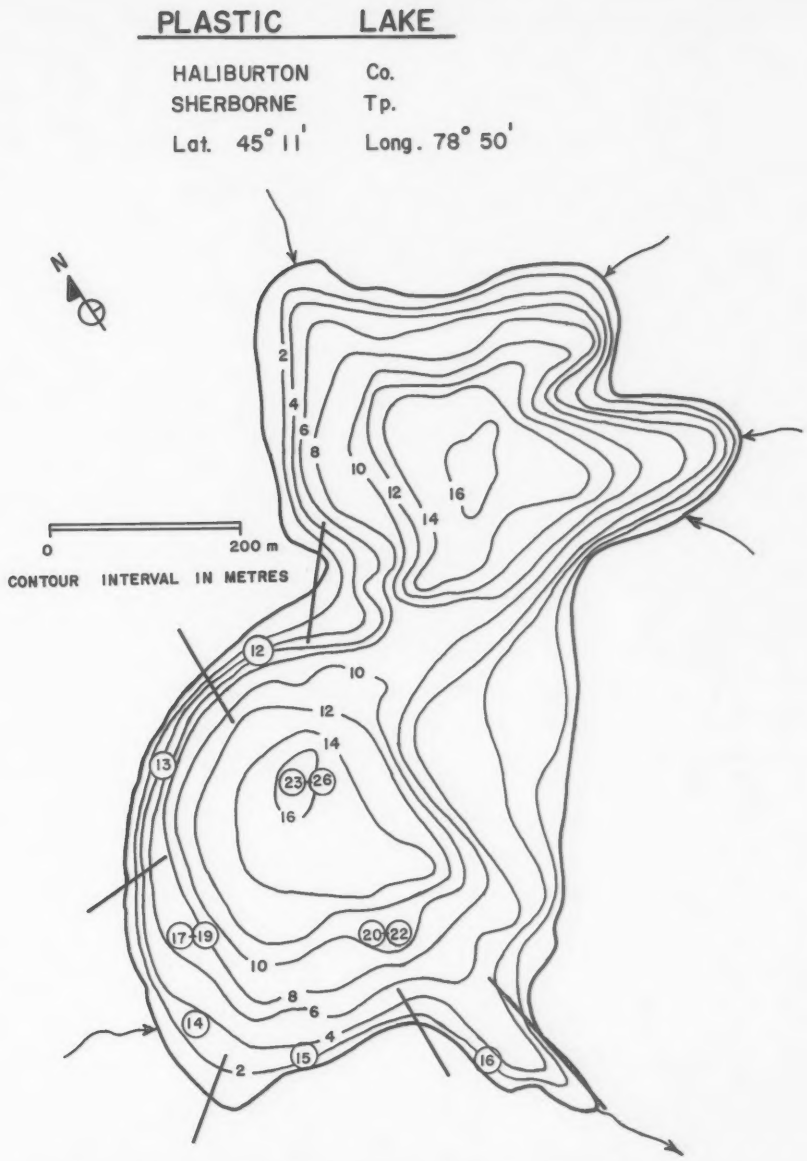

FIG. 1. Location of five littoral (sites 12–16) and three pelagial (sites 17, 20, and 23) sampling stations in Plastic Lake for monitoring snowmelt chemistry during spring 1982. Deepwater samples were collected at sites 18, 19, 21, 22, 24, 25, and 26. Lines denote division of shorelength into areas assumed representative of the sampling site contained within each.

experiments (France and Stokes 1987). Although this is only a "first-generation" model, unencumbered by rigid satisfaction of a large set of tenets, we follow the belief of Christophersen et al. (1984), Seip et al. (1985), and Minns et al. (1986) that such models are worthwhile in their generation of hypotheses testable by further experimentation or observation, as well as forming the basis for development of more complete and realistic future models.

The dynamics of *H. azteca* populations (Cooper 1965) make them easily amenable to simulation modelling of the effects of mortality from acid snowmelt. The salient properties include the following: (1) populations are normally stable in terms of size and structure, (2) the spring population in Canadian lakes consists entirely of adults overwintering from the previous summer, (3) populations are often univoltine, and it is therefore essential for population survival that the cohort of young animals produced in midsummer exists long enough to mature and produce young, and (4) the number of eggs produced in

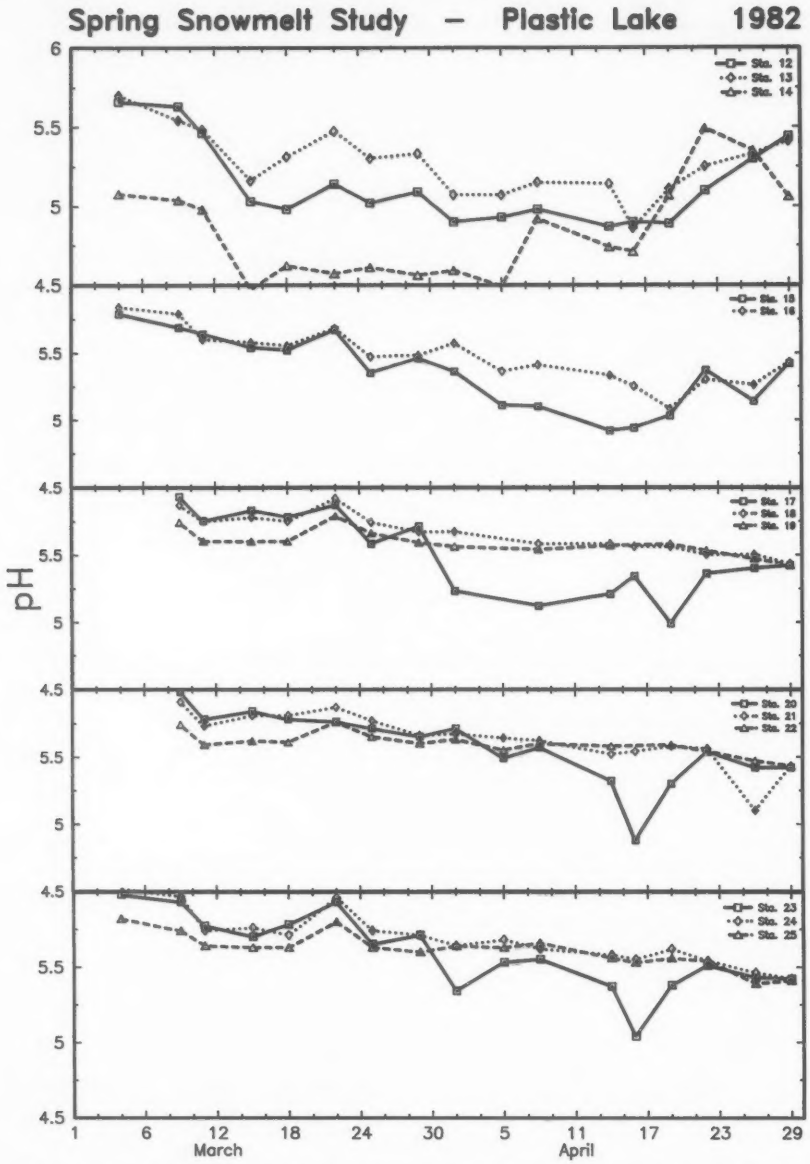

FIG. 2. pH versus time in spring of 1982 for stations 12–25. Graphs for sites 17, 20, and 23 also show pH data for the deeper stations (18–19, 21–22, and 24–25.

the population is a direct negative function of the mortality rate of the adults. For all these reasons, mortality may ultimately be as important a variable as either growth or reproduction in influencing population demography (Cooper 1965).

Model development in this study involved three phases. (1) As a result of the extremely low abundance or possible extinction of *H. azteca* presently in Plastic Lake, for modelling purposes, a population had to be empirically predicted in relation to the limnological characteristics of the lake. Data on the relationship of amphipods in nearby lakes to macrophyte density, total aqueous phosphorus concentration, and water depth were used to construct a theoretical preacidified *H. azteca* population in Plastic Lake, upon which to run the simulations. (2) Results of laboratory experiments on the toxicity of low pH to *H. azteca* (France and Stokes 1987) were integrated with existing chemical data on the fluctuations of acidic meltwater observed around the periphery of Plastic Lake during spring 1982. This simulation produced a population size for the lake based on *H. azteca* sensitivity to changing concentrations of hydrogen ions. (3) Finally, in view of the abnormally low abundance of *H. azteca* measured in Plastic Lake during 1983 (Stephenson and Mackie 1986), we wished

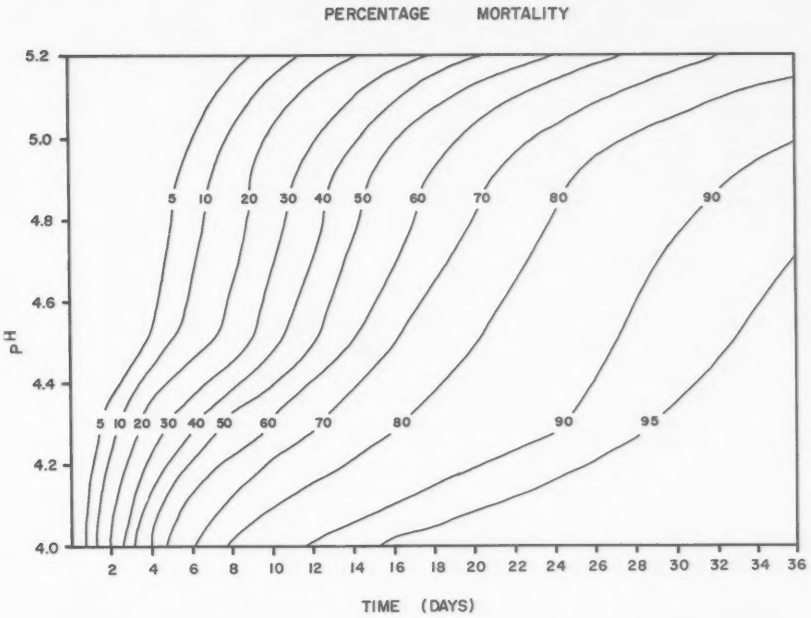

FIG. 3. Summary of mortality of adult *H. azteca* exposed to constant concentrations of low pH. Data are from France and Stokes (1987).

to evaluate how many years or generations of exposure to springmelt conditions of the type measured in 1982 it would take for our theoretical preacidification *H. azteca* population to decline to that level.

Data Base

Field Chemistry

Plastic Lake has several characteristics of a lake undergoing anthropogenic acidification; the major anion loading to the lake is anthropogenic sulphate, especially during the spring snowmelt period when the input acidity and aluminum levels are also maximum (LaZerte 1984; LaZerte and Dillon 1984). Biologically, the lake exhibits high benthic algal biomass (M. Jackson, Ontario Ministry of the Environment (MOE), unpubl. data) and relative dinoflagellate biomass in the plankton (K. Nicholls, MOE, unpubl. data) and depauperate or missing populations of the snail *Amnicola limosa* and two sensitive pisidiid clams (Rooke and Mackie 1984a, 1984b). Repeated winter–spring fish kills have also been reported (Harvey and Lee 1982).

To monitor the spatial and temporal variability in pH, eight accessible sites in the western half of Plastic Lake (Fig. 1) were selected. Five were shoreline sites (Nos. 12–16) situated in 1–2 m of water; they were sampled at 1 m depth. Another site was located at the deepest point in the lake and sampled at depths of 1, 2, 5, and 10 m (Nos. 23–26). The remaining two sites were situated halfway between the deep and shoreline stations in 5–10 m of water; they were sampled at 1, 2, and 5 m (Nos. 17–19 and Nos. 20–22). Samples were collected at all stations two or three times weekly during spring snowmelt 1982 until ice-out, after which surface mixing and turnover permitted the monitoring of only the stations at the single deep site (Nos. 23–26).

Water samples for pH and alkalinity measurements were pumped from the prescribed depths into a prerinsed, dark plastic container, allowing for at least two times overflow. The samples were returned to the laboratory, warmed to room temperature, and analyzed on the same day (Anonymous 1981).

For the annual variability component of this study, pH was measured in the major inflow and outflow to Plastic Lake from 1980 to 1984 (P. J. Dillon, MOE, Dorset Research Centre, unpubl. data) in a manner similar to that described above, but without the use of a pump.

Discharge and pH of the major inflow and outflow of Plastic Lake during this period are described in LaZerte (1984). The most prolonged and pronounced depression in pH (<4.5) (Fig. 2) was observed at the littoral site closest to the major inflow (No. 14). The pH at site 12 was depressed to 4.9. This site was just offshore of a major topographical convergence and was probably influenced more than the other sites by direct soil water seepage (LaZerte and Dillon 1984). The remaining littoral sites exhibited more gradual declines in pH with less pronounced minima. The openwater sites (Nos. 17–19, 20–22, and 23–26) had sharp pH depressions at the 1-m station only, with the lowest pH 5.0 being observed here at the very peak of the spring snowmelt. The stations at 2, 5, and 10 m exhibited only a very gradual pH decline down to 5.4 at ice-out and a subsequent increase during and after lake turnover.

Laboratory Toxicology

Experimental methods are described in detail in France and Stokes (1987). In summary, the following methodology was used: (1) aerated water from Paint Lake (pH 6.35, Ca 4.4 mg·

L^{-1}, and Al 0.03 mg·L^{-1}) was acidified and the pH maintained with H_2SO_4 for 8 d; (2) temperature ranged diurnally from about 12–18 to 20–25°C depending on the season; the influence of temperature on hydrogen ion toxicity in *H. azteca* appears minor (France and Stokes 1987); (3) 10–15 adult amphipods, provided with organic sediment as a food source, were used per treatment. The results used for the present model were selected from a series of five experiments involving *H. azteca* collected from four lakes, two of which are moderately acidic (Crosson, pH 5.65; Heney, pH 5.74; Blue Chalk, pH 6.47; Harp, pH 6.22). Data were pooled to produce the following testing concentrations with number of replicates in parentheses: pH 5.20 (6), pH 4.85 (10), pH 4.55 (16), pH 4.30 (10), and pH 4.05 (16). The mortality data were averaged for each treatment and then analyzed by graphical interpolation (Litchfield 1949) to obtain a series of percentile survival times. These summarized results are thought to represent an expected average response of *H. azteca* to acidification of softwater habitats in the Haliburton–Muskoka region.

The results are presented as mortality–time isopleths (Fig. 3). An acute effect develops below pH 4.3, and for purposes of the model, no mortality is assumed above pH 5.2. Survivorship curves and calculation of median survival times and LC50s are presented in France and Stokes (1987).

Model Description and Results

Characterization of a Theoretical *H. azteca* Population for Plastic Lake

A theoretical *H. azteca* population in Plastic Lake was empirically derived based on (a) macrophyte density, (b) total phosphorus concentration, and (c) amphipod depth distribution.

(a) The demonstrated close association of *H. azteca* with macrophytes (e.g. Lindeman and Momot 1983) is due to the combined effect of the plants as both a refugium from fish predation (Johannes and Larkin 1961) and as a substrate for epiphytic algae, an important food source (Hargrave 1973). In nearby Blue Chalk Lake the density of adult *H. azteca* (D_H in number per square metre) during spring 1984 was directly related to macrophyte biomass (M_B in grams dry weight per square metre) as sampled with a 45.6-cm^2 cover:

$$(1)\quad D_H = 174.5 + 1.73\,M_B \quad (r = 0.89,\ n = 33).$$

A series of 30 macrophyte samples, 6 in each of the areas sampled for chemistry, were taken from the littoral zone of Plastic Lake in July 1985 with the same corer. Following oven drying at 60°C for 48 h and subsequent weighing, the values of plant biomass were used to predict (cf. Downing 1986) potential densities of *H. azteca* per sample from equation (1) developed for Blue Chalk Lake.

(b) The average density of adult amphipods in Haliburton–Muskoka lakes during spring 1984 (R. L. France, unpubl. data) was also related to system productivity as reflected by lake total phosphorus concentration (TP in micrograms per litre) from mean ice-free season values (P. J. Dillon, unpubl. data):

$$(2)\quad \text{Average } D_H/\text{g } M_B = -0.58 + 0.32\text{TP} \quad (r = 0.84,\ n = 7 \text{ lakes}).$$

Lakes of higher productivity can support a higher density of *H. azteca* per unit macrophyte biomass. Inserting the TP concentration of 6.7 μg·L^{-1} for Plastic Lake into equation (2) produced a predicted density of 1.56 amphipods·g macrophyte biomass^{-1} for the lake. Because of the oligotrophic nature of Plastic Lake, this ratio is toward the lower end of the range measured for other Dorset lakes (1.01–4.54). By comparison, the density ratio for Blue Chalk Lake was directly determined to be 2.49 ± 0.45 (SE) (n = 12), a difference of 37.4%. All densities derived from equation (1) developed for Blue Chalk Lake were therefore reduced by the interlake correction factor from equation (2). Thus the theoretical densities (number per square metre ± SE — reflecting variability in macrophyte biomass) calculated for the littoral sites were as follows: site 12, 316 ± 33; site 13, 301 ± 29; site 14, 863 ± 124; site 15, 321 ± 12, and site 16, 506 ± 105.

TABLE 1. Abundance (in hundred thousands) of areally weighted theoretical population of *H. azteca* in Plastic Lake.

	Littoral site				
Depth (m)	12	13	14	15	16
≤1.0	7.6	4.8	40.7	8.2	11.5
1.0–2.0	2.2	1.4	11.6	2.3	3.3
>2.0	1.1	0.7	5.8	1.2	1.6

(c) In Dorset lakes, as elsewhere in Canada (Biette 1969; Mathias 1971; Lindeman and Momot 1983), the distribution of *H. azteca* changes with depth (R. L. France, unpubl. data). On average, 70% of the population is found in water ≤1.0 m, 20% between 1.0 and 2.0 m, and the remaining 10% almost entirely between 2.0 and 3.0 m. From the deepest point in the lake, near pelagial site 23 (Fig. 1), a line was drawn on the map bisecting the shoreline between littoral sampling sites into two equal lengths. The areas contained within the 1.0-, 2.0-, and 3.0-m contours were then determined for each littoral site and multiplied by the appropriate densities corrected for depth. This gave a weighted population size estimate of 10.4×10^6 amphipods for the region of Plastic Lake covered by chemical sampling (Table 1).

Integration of Field Chemical and Laboratory Toxicological Data, and Simulations for 1982

The acidic pulses were converted into a stepwise form and the time spent at each pH (rounded to the nearest 0.05 unit) was determined. By referring to Fig. 3 this duration allowed us to calculate percentage mortality of amphipods. Then, using the empirically predicted density for each site as the initial value, and following the pH depression through its time course, the increment mortality was calculated for each of the five littoral sites sampled in 1982 (e.g. Fig. 4). These mortalities ranged from 3.0% at site 16 near the outflow to 77.2% at site 14 near the inflow (Table 2).

Pulses of acidic meltwater are rarely monitored throughout the littoral zone. More frequently they are normally gauged from midlake sampling locations or inferred from the more accessible inflows and outflows. To determine whether such data would produce very different predicted results, the expected mortalities at the three pelagic sites were assessed using midlake pH values and assuming an initial density of 461 amphipods·m^{-2}, the lake average. The total mortality at

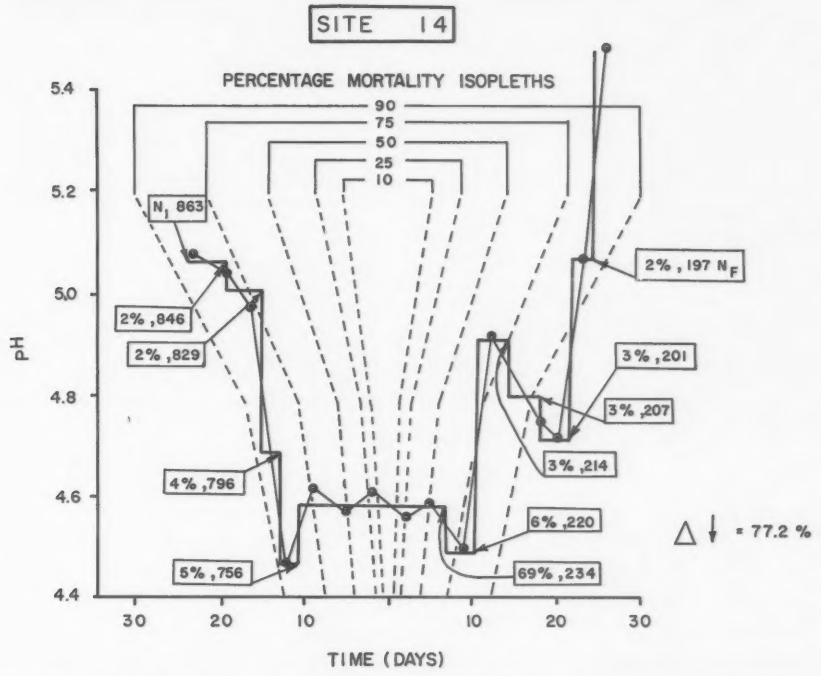

FIG. 4. Evaluation of lethality of an acid pulse to *H. azteca*. Broken lines denote the time required to produce 10, 25, 50, 75, and 90% mortality of adult amphipods by pH determined from laboratory work. This provides a rough approximation of the total expected mortality, i.e. between 75 and 90% for this example. Solid line represents the temporal fluctuation of pH observed at site 14 in Plastic Lake, March–May 1982. Mortality of amphipods would be expected if observed pH fluctuation overlaps the laboratory curves. The acid pulse has been converted to stepwise form and the percentage mortality at each stage calculated from the duration of time spent at particular pH concentration from Fig. 3. The population density resulting from the cumulative effects of this mortality is indicated. Assuming an empirically derived initial density of 863 amphipods·m^{-2}, the resulting final density of 197·m^{-2} indicates a total mortality of 77.2%.

1.0 m depth for the three pelagial sites was estimated to be much lower, 3.1–8.7% (Table 2). The depression of pH at the 2.0- and 3.0-m sampling depths was not great enough to produce any mortality. The effects of episodic pH depressions measured in the inflow and outflow of Plastic Lake during spring 1982 (fig. 8 and 9 in LaZerte 1984) were also simulated using initial densities from the littoral sites closest to the streams. The mortalities were 98.3 and 10.7%, respectively (Table 2).

Because of the reduced severity of pH fluctuation below 1.0 m, *H. azteca* below this depth will not decline from acid-induced mortality in our simulation. The areally weighted population size following simulated exposure to the littoral acid pulses was 6.7×10^6, a drop of 35.2% from the total of Table 1. This simulation suggested, therefore, that the magnitude and duration of the depressions of pH measured in the littoral zone in Plastic Lake during 1982 were severe enough to reduce *H. azteca* population size.

Time Trend Simulation

Although littoral chemistry data exist for only 1982, the chemistry of the inflow and outflows of Plastic Lake have been measured from 1980 to 1984 (P. J. Dillon, unpub. data). The pH depressions in the spring of 1982 were the severest on record. Assuming that the ratio of mortality predicted from the pH depressions in the inflow and outflow to that from the five littoral sites in 1982 were the same for the other years, a 5-yr averaged population mortality was estimated to be 29.7%.

The predicted absence of acid-induced mortality in the 30% of the *H. azteca* population inhabiting water below 1.0 m depth indicates that these animals may be able to restock the shallower habitats depleted by low pH, and in a sense "buffer" the rate of littoral population decline. It is assumed that every spring the more favourable littoral conditions in terms of temperature, food supply, and macrophyte biomass will attract these amphipods from deeper water where they are normally intraspecifically segregated (Mathias 1971). The population will then redistribute itself in accordance to the previously determined ratio of depth distribution. Therefore, with every iteration of the model, 70% of the total population (that which inhabits shallow water $\leq$1.0 m) is reduced 29.7% by direct acid mortality, while the remaining 30% of the total population (in deeper water >1.0 m) is subsequently reduced through emigration to the shallow zone (Fig. 5). This time trend simulation assumes that the acid mortality is additive, not compensatory, and that the declining population maintains a balanced

TABLE 2. Model-derived incremental mortality of *H. azteca* from pulses of acid snowmelt measured during March–May 1982 in Plastic Lake (Fig. 2). Sites 12, 13, 15, and 16 are littoral stations; sites 17, 20, and 23 are 1.0-m depths at pelagic stations (Fig. 1); outflow and inflow data are from LaZerte (1984). d = days; % = percentage mortality calculated from laboratory data (Fig. 3); Δ ↓ = percentage population decline at pulse termination from difference between initial and final densities.

Site 12				Site 13				Site 5				Site 16				Site 17				Site 20				Site 23				Inflow			
pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}	pH	d	%	No.·m^{-2}
			316				301				321				506				461				461				461				863
5.00	6	5	300	5.20	2	2	295	5.10	5	4	308	5.15	1	1	501	5.20	3	2	452	5.20	2	2	452	5.20	1	1	456	4.75	5	5	820
5.15	4	1	297	5.05	6	5	280	5.00	4	3	299	5.05	2	1	496	5.10	4	3	438	4.90	1	1	447	5.05	1	1	452	4.85	1	1	812
5.00	3	2	291	5.15	8	6	263	4.95	4	4	287	5.20	1	1	491	5.20	4	3	425	5.20	1	1	443	5.20	1	1	447	4.70	2	2	795
5.10	4	3	283	4.85	3	3	256	5.05	3	2	281				·	5.00	2	1	421	5.10	2	2	434				·	4.80	2	2	780
4.90	7	10	254	5.05	2	2	250				·				·				·				·				·	4.40	2	4	748
4.95	6	7	236	5.20	1	1	248				·				·				·				·	Δ ↓			·	4.65	4	4	718
4.90	9	20	189				·				·				·				·				·				2.9%	4.50	8	26	532
5.10	3	2	185				·				·				·				·				·					4.45	12	55	239
			·				·				·				·				·				·		Outflow			4.30	6	40	144
			·				·				·				·				·				·					4.35	6	33	96
			·				·				·				·				·				·				506	4.30	4	24	73
Δ ↓			41.4%	Δ ↓			17.6%	Δ ↓			12.5%	Δ ↓			3.0%				·				·	5.20	2	2	496	4.35	7	40	44
																			·				·	4.90	5	7	461	4.40	11	55	20
																			·				·	5.20	2	2	452	4.50	7	20	16
																			·				·					4.60	5	7	15
																			·				·								
																Δ ↓			8.7%	Δ ↓			5.9%	Δ ↓			10.7%	Δ ↓			98.3%

and unaltered ratio of birth rate to death rate (cf. Cooper 1965).

The simulation suggests that it would take 20 yr of springmelt-induced mortality before the population decreased to a density of $<1 \cdot m^{-2}$. The median survival time for this rate of decline is 4 yr. If the deeper water amphipods did not emigrate, the simulated decline would assume a simple exponential function with shallow water densities of $<1 \cdot m^{-2}$ achieved within a decade and a median survival time for this subpopulation of about 2 yr.

Discussion

Model Assumptions

The assumptions used in the present simulation, because of its uncomplicated design, are really no different from those implicit in laboratory toxicological work when attempts are made to extrapolate the experimental results to natural situations.

Factors not included in the simulation model, but which could potentially exacerbate the decline of a *H. azteca* population exposed to lake acidification, include the following.

(1) Interaction with metals. Pulses of acidic snowmelt in Plastic Lake are characterized by greatly elevated concentrations of inorganic monomeric Al, Mn, and reduced concentrations of Ca (LaZerte 1984; unpubl. data), all of which can intensify the stress experienced by certain crustaceans at low pH (Havas and Likens 1985). Although the present model considers only the effects of increased H^+ on a *H. azteca* population, this is believed, by far, to be the dominant agent toward amphipod toxicity (R. L. France and P. M. Stokes, unpubl. data).

(2) Stress memory. When calculating incremental lethality throughout the acidic pulse, it is assumed that the mortality response of *H. azteca* to low pH remains unaltered by history of exposure prior to that stage of the pH fluctuation. But while *H. azteca* cannot acclimate to lethal levels of pH, they do display a reduced capability to tolerate such concentrations following preexposure to sublethal values, i.e. they apparently develop a physiological "memory" of stress at low pH and become sensitized (France and Stokes 1987). The total mortality of a *H. azteca* population exposed to any acidic pulse would therefore be expected to be higher than our conservative model would suggest.

(3) Long-term life cycle effects. The model predicts the decline of a theoretical population of *H. azteca* solely through direct mortality from inputs of acid snowmelt and disregards more subtle effects of long-term acidification on reproduction although some evidence suggests that *H. azteca* populations may display anomalous reproductive patterns in lakes with pHs approximating 5.6 (Stephenson and Mackie 1986).

Factors not considered by the model which could mitigate the effects of acidic snowmelt include the following.

(1) Avoidance. Amphipods avoid acidic water combined with elevated concentrations of CO_2 or Cu (Costa 1967; de March 1983), suggesting that they could escape mass mortality during the period of springmelt by seeking refuge in the profundal zone or by burrowing into littoral sediments. The former has been shown for zooplankton (Groterud 1972).

(2) Acclimatization or natural selection. The time trend simulation assumes an unaltered physiological response of *H. azteca* to continuing acidification. France and Stokes (1987), however, found population variation in the resistance of *H.*

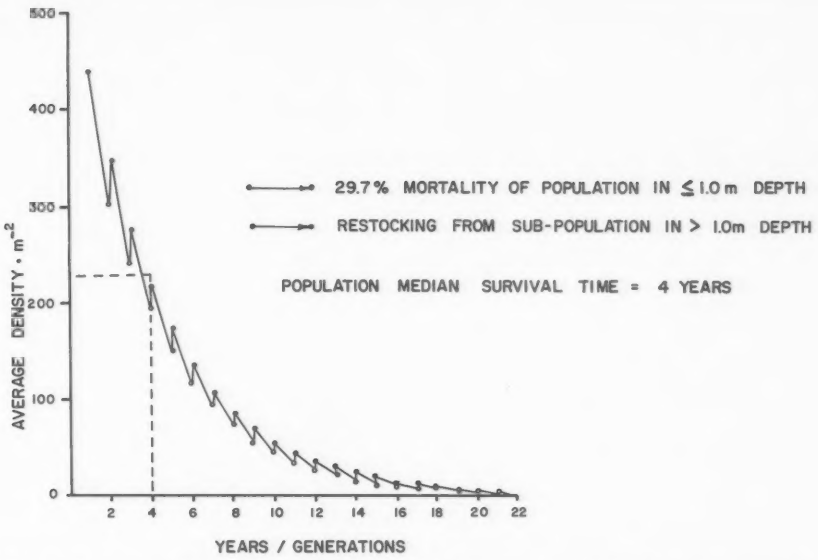

FIG. 5. Scenario predicting the decline of a theoretical *H. azteca* population from acid snowmelt toxicity. The model assumes that the mortality caused by low pH is additive to that already naturally acting on the population and that a balanced and unaltered ratio of birth rate to death rate exists at all density levels. Time at which 50% mortality occurs is indicated.

azteca to low pH; amphipods from two acidifying lakes were able to resist lethal concentrations of H^+ significantly longer than those collected from circumneutral lakes.

(3) Population compensatory mechanisms. *Hyalella azteca* inhabiting lakes in the Haliburton–Muskoka region exhibit a high degree of stability in annual density (R. L. France and P. M. Stokes, unpubl. data). It is possible that because *H. azteca* populations may already be adjusted to compensate for high rates of natural mortality (Cooper 1965), some of the mortality from low pH springmelt may never cause a decrease in abundance. Alternatively, if the acid mortality is additive as the model assumes, then populations may in turn compensate for loss of older animals with an increased birth rate (Nicholson 1957; McFarlane and Franzin 1978).

(4) Macrophyte buffering. The production of alkalinity through normal processes of primary production within the macrophyte bed (Hutchinson 1975) could reduce the severity of low pH pulses to *H. azteca* capable of taking advantage of this favourable microhabitat.

Other variables not considered in the model whose effects on rate of population decline are unclear include secondary effects of lake acidification on predators, food supply and macrophyte habitat, and especially the complex spatial heterogeneity of acid inputs throughout the littoral zone of Plastic Lake.

Because of both the biological assumptions and absence of more detailed chemical mapping of snowmelt inflows, we considered it inappropriate to generate confidence limits about our predictions (although this was possible), as these could be interpreted as promoting a false sense of security in what is put forward as essentially a speculative exercise. However, despite the fact that several ecological and physiological variables were not included, the current model should be viewed as a first approximation in an iterative process.

Model Implications

Ultimately, construction of the theoretical *H. azteca* population in Plastic Lake should be regarded as nothing more than a tool upon which to run the simulation models. The time trend simulation, i.e. whether elimination occurs within 10 or 20 yr, should be viewed as secondary to the major result — that the acid pulses currently observed within Plastic Lake today *do* have the potential to kill *H. azteca*. Based on simulations from 1982 which produced an areally weighted mortality of 35.2%, the present model strongly suggests that population decline of *H. azteca* within anthropogenically acidified lakes can be explained by direct mortality resulting from short-term pH fluctuations. This is similar to what is believed for *Mysis relicta* (Nero and Schindler 1983), but contrary to that hypothesized for either crayfish or molluscs (France 1984; Servos 1983).

The model suggested that mortality in the lake may be site specific. Due to their close association with macrophytes, the littoral distribution of *H. azteca* is not random. Because the greatest biomass of macrophytes may often occur near the mouths of inflows in these lakes (Hitchin et al. 1984), the location where the pH depressions are monitored will greatly influence conclusions regarding the severity of their effects on lake biota. For example, the average mortality estimated from the three pelagic sites was less than a quarter of that predicted from the littoral stations. Likewise, the mortality calculated from the outflow data, often regarded as an integration of the lake whole (Goldstein et al. 1984), underestimated littoral mortality by 66%.

Test of Model Predictions

The *H. azteca* population in Plastic Lake is at the limit of detection, and may even be eliminated. Rigorous sampling

during the ice-free months of 1983 yielded a density of $8 \cdot m^{-2}$ compared with average densities of about $500-1000 \cdot m^{-2}$ for five other Haliburton–Muskoka lakes (Stephenson and Mackie 1986). In 1984, the density of *H. azteca* in Plastic Lake was about $5 \cdot m^{-2}$ (M. Stephenson, University of Guelph, Guelph, Ont., pers. comm.), and with collection of over 200 Ekman grab samples throughout the littoral zone in spring 1985, the estimated density was $1 \cdot m^{-2}$ (G. Krantzberg, University of Toronto, Toronto, Ont., pers. comm.). In a series of 30 samples taken in areas of densest macrophyte cover in July 1985 for this study, we found no amphipods at a time when densities within similar habitats in nearby lakes were between 1064 and $2896 \cdot m^{-2}$ (mean of 5–13 amphipods per sample).

With a starting *H. azteca* density of $8 \cdot m^{-2}$, our simulation model (Fig. 5) would predict that densities of $<1 \cdot m^{-2}$ would be achieved within 4–6 yr. In contrast, field observations indicated that this decrease in abundance took only 2 yr. This supports Stephenson and Mackie's (1986) contention, although it was based on only a few specimens, that recruitment may have also been severely stressed in Plastic Lake during 1983.

The lack of suitable benthic habitats in many moderately coloured, acidic lakes may be an important variable precluding the occurrence of *H. azteca* in such waters (R. L. France and P. M. Stokes, unpubl. data). Plastic Lake, however, contains abundant growths of macrophytes (Hitchin et al. 1984), which together with the absence of large-sized populations of predaceous fish (H. Harvey, University of Toronto, Toronto, Ont., unpubl. data) suggests that it would support a large *H. azteca* population, low pH notwithstanding. Plastic Lake does contain a population of *Crangonyx richmondesis laurentianus*, an amphipod characteristic of "bog ponds and small typically acidic lakes" (Bousfield 1958). Although *C. richmondesis* can occur in reduced abundance together with *H. azteca* (Mathias 1971), and certainly *H. azteca* does occur separately, the presence of *C. richmondesis* without *H. azteca* (Sprules 1967) may provide additional support of acidified conditions (midsummer pHs <6.0) which are indicated by the absence of *H. azteca* (Stephenson and Mackie 1986) alone.

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Assessment of Pacific Skipjack Tuna (*Katsuwonus pelamis*) Resources by Estimating Standing Stock and Components of Population Turnover from Tagging Data

P. Kleiber

Southwest Fisheries Center, P.O. Box 271, La Jolla, CA 92038, USA

A. W. Argue

Pacific Coast Bio-Resources, 3-3142 Cedar Hill Rd., Victoria, B.C. V8T 3J6

and R. E. Kearney

Fisheries Research Institute, P.O. Box 21, Cronulla NSW 2230, Australia

Kleiber, P., A. W. Argue, and R. E. Kearney. 1987. Assessment of Pacific skipjack tuna (*Katsuwonus pelamis*) resources by estimating standing stock and components of population turnover from tagging data. Can. J. Fish. Aquat. Sci. 44: 1122–1134.

More than 140 000 tagged skipjack tuna (*Katsuwonus pelamis*) were released during 3 yr over a large portion of the central and western Pacific. Tag returns exceeded 6000. We developed a set of tag attrition models to analyze tag release and return data and catch and effort statistics for the study area. We used these models to assess the status of the skipjack resource in the whole study area and within subdivisions thereof. Total standing stock was estimated at 3 million metric tons (Mt), (95% confidence range 2.5–3.7 Mt). Overall attrition rate (including losses to natural mortality, fishing mortality and emigration) was $0.17 \cdot mo^{-1}$ ($0.15–0.20 \cdot mo^{-1}$). Total throughput was estimated at $6.2\ Mt \cdot yr^{-1}$ ($5.5–7.1\ Mt \cdot yr^{-1}$) compared with catch of $<0.3\ Mt \cdot yr^{-1}$. Overall harvest ratio was 0.04. Harvest ratios for seven subareas for which detailed catch and effort statistics were available ranged from 0.02 to 0.46; only one exceeded 0.17. Low harvest ratios over most of the study area during the period tags were at large imply a potential for increased skipjack catches in many subareas and in the whole study area.

Plus de 140 000 bonites à ventre rayé (*Katsuwonus pelamis*) marquées ont été relâchées dans une zone couvrant une grande partie du centre et de l'ouest du Pacifique au cours d'une période de 3 ans. On a pu récupérer plus de 6 000 étiquettes. Les auteurs ont élaboré une série de modèles d'attrition des étiquettes pour l'analyse des données sur l'étiquetage et le retour des étiquettes et des statistiques de prises et d'effort de pêche dans la zone étudiée. Les modèles ont servi à évaluer l'état de la ressource en bonites dans l'ensemble et les subdivisions de la zone d'étude. Le stock présent a été estimé à 3 millions de tonnes métriques (Mt) (niveau de confiance à 95 % de 2,5–3,7 Mt). Le taux d'attrition total (comprenant les pertes par mortalité naturelle, pêche et émigration) a été estimé à $0{,}17 \cdot mo^{-1}$ ($0{,}15–0{,}20 \cdot mo^{-1}$). La productivité totale a été estimée à $6{,}2\ Mt \cdot an^{-1}$ ($5{,}5–7{,}1\ Mt \cdot an^{-1}$) pour des prises inférieures à $0{,}3\ Mt \cdot an^{-1}$. Le rapport de récolte total était de 0,04. Les rapports de récolte de sept sous-zones pour lesquelles on disposait de statistiques détaillées sur les prises et l'effort de pêche se situaient entre 0,02 et 0,46 et un seul était supérieur à 0,17. Les faibles rapports de récolte pour la plus grande partie de la zone étudiée au cours de la période pendant laquelle les poissons marqués étaient en haute mer supposent une possibilité d'accroître les prises de bonites dans bon nombre de sous-zones de même que dans l'ensemble de la zone d'étude.

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Annual skipjack tuna (*Katsuwonus pelamis*) catches from the area of the South Pacific Commission increased rapidly from less than 5000 metric tons (t) in the early 1960's to approximately 220 000 t in the early 1980's. Skipjack are a short-lived, fast growing, highly fecund species distributed throughout all tropical and subtropical ocean waters. Their lifestyle is apparently quite suitable for supporting a high harvest rate. Nevertheless, with increased catches, many countries in the region became concerned that interactions among surface fisheries might be sizeable and that increased yields might not be sustainable. The Skipjack Survey and Assessment Programme was undertaken by the Commission to assess the status of the skipjack resource and its ability to support this increased fishing pressure. Tagging was adopted as the principal stock assessment technique (Anonymous 1975). Between October 1977 and August 1980 the Skipjack Programme tagged and released approximately 140 000 skipjack throughout and beyond the area of the South Pacific Commission (Fig. 1). Over 6000 of these tagged fish were recaptured and reported to the Commission.

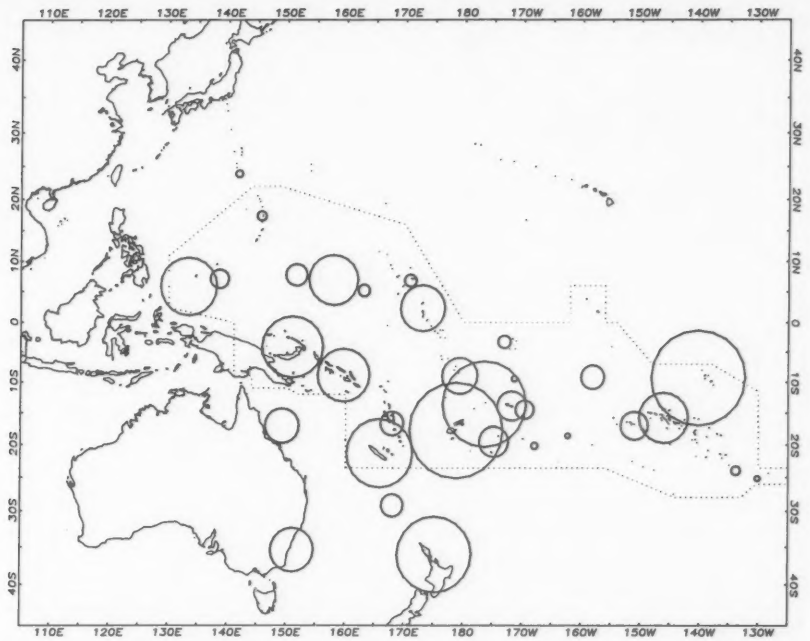

FIG. 1. Distribution of tag releases (circles) and boundaries of the South Pacific Commission Region (dotted line). The circles are centered on each subarea in which tags were released. The areas of the circles are proportional to the number of tagged skipjack released.

We present an analysis of tag release and recovery data for the purposes of assessing the standing stock of skipjack, rate of renewal (turnover) of the skipjack resource, and current levels of fishing pressure on the skipjack resource in the region as a whole and in the waters of individual countries and territories for which detailed catch and fishing effort data were available. For this purpose we developed a set of closely related analytical models which, using tagging data and catch and effort statistics, gives estimates and confidence limits for various parameters useful in defining the status of a population that is supporting a fishery. Some of these parameters are familiar: standing stock, natural mortality, and fishing mortality. Others are not so familiar but are useful in the context of this widely distributed and mobile species. We define these as follows.

Attrition: rate of loss of standing stock expressed as proportion of standing stock. It encompasses all loss factors including natural mortality, fishing mortality, emigration, and growth out of vulnerability to the fishery.

Throughput: product of attrition and standing stock; a measure of the passage of biomass through the stock. It encompasses death, emigration, and growth out of the vulnerable size class. Under steady-state conditions, it is also the in situ productivity plus immigration of vulnerable-sized individuals.

Harvest ratio: ratio of catch rate to throughput (equivalent to the ratio of fishing mortality to attrition). If fishing mortality is small relative to the population turnover, i.e. the harvest ratio is low, it is likely that fishing is having little impact on the population.

Tagging and Tag Recovery Methods

Tagging was carried out over a period of 3 yr in three 10-mo cruises each using one chartered Japanese pole-and-line vessel. The itinerary of the tagging cruises covered the whole study area, which includes the area of the South Pacific Commission and some adjacent waters where skipjack were known to be abundant (Kearney 1982b). The number of tags released in each area was not uniform (Fig. 1) because tagging success depended on fishing conditions, which were quite variable in space and time.

Skipjack were captured by pole-and-line fishing. The fish were poled onto tagging cradles where they were measured and tagged with plastic dart tags according to the technique described by Kearney and Gillett (1982). Fishermen on local and foreign-based vessels and workers at processing facilities were the primary sources of returned tags. Locally based fisheries within the study area were the pole-and-line operations in Papua New Guinea, Solomon Islands, Fiji and Palau, the *alia* fishery in Western Samoa, the *bonitier* fishery in the Society Islands of French Polynesia, and the purse-seine fishery in New Zealand. Information on local fisheries is contained in the final reports to the individual countries by the Skipjack Programme (e.g. Kearney 1982a; Argue and Kearney 1982, 1983; Gillett and Kearney 1983; Kleiber and Kearney 1983). Foreign fleets taking significant quantities of skipjack at the time most tags were at large were the long-range Japanese pole-and-line fleet and steadily increasing Japanese and United States purse-seine fleets.

Rewards were given and lotteries conducted to encourage return of tags (Kearney 1982b). It was possible to check efficiency of part of the tag return system with a tag-plant experiment in which 131 fish from the holds of purse-seiners were tagged and replaced in the holds by New Zealand Ministry of Agriculture and Fisheries personnel during the 1980–81 New

Zealand fishing season.

To investigate tag shedding and mortality due to tagging, a double tagging experiment was carried out in the waters of Fiji in 1980, during which 5399 double tagged skipjack were released, interspersed with 5626 single tagged fish (Skipjack Programme 1981). A further experiment was conducted with skipjack held at a research facility of the United States National Marine Fisheries Service at Kewalo Basin, Honolulu. Sixteen captive skipjack were tagged with Skipjack Programme tags and were observed for 7 wk along with 14 untagged controls (R. E. Kearney, unpubl.).

Analytical Methods

We assessed population parameters by analyzing plots of tag return rate (number of tags returned per unit time) against time at large. The tag return rate is expected to decrease with time because tag density in the fished populations should decline due to a variety of factors (e.g. mortality, emigration, tag shedding). The analyses described in this report were performed with a set of models in which tag return rates are predicted as a function of time from release and in which variations in exploitation are taken into account. The choice of model depends on which parameters are to be estimated and on whether catch data or effort data are used.

Derivation of Models

Immediate mortality and shedding have been defined as type 1 losses (Beverton and Holt 1957, p. 201; Bayliff and Mobrand 1972). These losses reduce the effective number of tagged fish at large at time zero. Thus if N_0 fish are tagged and if α is type 1 survival, then the effective number of tagged fish at the start is αN_0.

Following type 1 losses, a number of other factors reduce the population of tagged fish. Factors that affect all fish include natural mortality, emigration, fishing mortality, and growth out of vulnerability to the fishery. In addition, the population of tagged fish can undergo what is called type 2 or long-term loss by tag shedding and extra mortality due to carrying a tag. If, following type 1 losses, all attrition factors operate such that the tagged population decreases exponentially, then the number of tagged fish at large as a function of time, t, following tagging is

$$(1)\quad N = \alpha N_0 e^{-(Z+\psi)t}$$

where Z = total attrition rate for a group of untagged fish (time^{-1}) and ψ = additional attrition for tagged fish (time^{-1}). The rate at which usable tags are returned is given by

$$(2)\quad \frac{\partial r}{\partial t} = \beta F N = \alpha N_0 e^{-(Z+\psi)t}$$

where r = cumulative number of usable tag returns, F = fishing mortality (time^{-1}), and β = proportion of recaptured tags that are actually returned with usable recapture information. (Not all tag returns could be used in the analysis because some had unknown or imprecisely known times of recapture.)

We assume that ψ and β are constant during the time that tags are recovered. We also assume that Z is constant even though F, which is a component of Z, is likely to vary considerably with changes in effort expended by the fishery. For $Z \gg F$, this assumption in not bad, and later we will relax this assumption.

Defining F_i to be the average fishing mortality during the ith time unit following tagging and integrating Equation 2 under the assumption of constant attrition gives

$$(3)\quad r_i = \frac{\alpha\beta N_0 F_i}{(Z+\psi)} e^{-i(Z+\psi)} \left[e^{(Z+\psi)} - 1\right]$$

where r_i is the number of usable tag returns in time unit i. Equation 3 is a general model from which particular models were derived based on the following considerations.

The unknown fishing mortalities, F_i, can be approximated by

$$(4)\quad F_i \simeq q f_i \simeq \frac{c_i}{P}$$

where c_i = catch in biomass units in time unit i, P = standing stock in biomass units (assumed constant in time), q = catchability coefficient, or fraction of the standing stock harvested by one unit of fishing effort, and measured in inverse units of fishing effort (assumed constant in time), and f_i = units of fishing effort in time unit i.

Thus, either catch data or effort data can be used depending on which term in Equation 4 is substituted for F_i in Equation 3. Whether catch data or effort data are used influences the parameter estimates. Therefore, parameters that were estimated both ways are differentiated by a subscript, c, for parameters in models using catch data and a subscript, f, for parameters in models using effort data.

To complete the derivation of particular models, the following equations were substituted into the general model. The throughput (biomass per unit time) is given by

$$(5)\quad T = Z_c P.$$

Fishing mortality is not treated as constant over all time intervals, but a measure of the average fishing mortality can be obtained. If we have an average catch rate, $\bar{c}$, or an average effort rate, $\bar{f}$, then the average fishing mortality is given by

$$(6)\quad F_c = \frac{\bar{c}}{P};\ F_f = q\bar{f}.$$

The harvest ratio (unitless) is then given by

$$(7)\quad H_c = \frac{F_c}{Z_c};\ H_f = \frac{F_f}{Z_f}.$$

The resulting set of models is detailed in Table 1 wherein each equation, except for the last, has two parameters relevant to stock assessment which are confounded with α, β, and ψ, which we will call nuisance parameters.

The last model in Table 1 is an exception. All others allow the fishing mortality to vary with time, but contain the paradoxical assumption that the attrition rate is constant. When catch is used, this inconsistency is difficult to correct because standing stock should vary if the attrition varies. The model would therefore need to incorporate input and output of untagged fish with an attendant list of further assumptions.

However, when effort is used, it is logically consistent to allow to varying attrition rate and still assume a constant catchability. Defining M = attrition rate of untagged fish exclusive of fishing mortality (time^{-1}), we can substitute $M + F + \psi$ for Z in Equation 2. Then substituting qf_i for F and integrating leads to the last equation in Table 1. In this model the quantity $\alpha\beta$ is no longer confounded with other parameters. Therefore in theory this model could be used to estimate $\alpha\beta$. Note that the effort must vary for this to work because if f_i is constant, this

TABLE 1. Set of models used for estimating population parameters.

Parameters	Model
Z_c P	$r_i = \frac{\alpha\beta N_0 c_i}{P(Z_c+\psi)} e^{-i(Z_c+\psi)}\left[e^{(Z_c+\psi)} - 1\right]$
Z_f q	$r_i = \frac{\alpha\beta N_0 q f_i}{(Z_f+\psi)} e^{-i(Z_f+\psi)}\left[e^{(Z_f+\psi)} - 1\right]$
Z_c T	$r_i = \frac{\alpha\beta N_0 Z_c c_i}{T(Z_c+\psi)} e^{-i(Z_c+\psi)}\left[e^{(Z_c+\psi)} - 1\right]$
Z_c F_c	$r_i = \frac{\alpha\beta N_0 F_c c_i}{\bar{c}(Z_c+\psi)} e^{-i(Z_c+\psi)}\left[e^{(Z_c+\psi)} - 1\right]$
Z_f F_f	$r_i = \frac{\alpha\beta N_0 F_f f_i}{\bar{f}(Z_f+\psi)} e^{-i(Z_f+\psi)}\left[e^{(Z_f+\psi)} - 1\right]$
Z_c H_c	$r_i = \frac{\alpha\beta N_0 Z_c H_c c_i}{\bar{c}(Z_c+\psi)} e^{-i(Z_c+\psi)}\left[e^{(Z_c+\psi)} - 1\right]$
Z_f H_f	$r_i = \frac{\alpha\beta N_0 Z_f H_f f_i}{\bar{f}(Z_f+\psi)} e^{-i(Z_f+\psi)}\left[e^{(Z_f+\psi)} - 1\right]$
$\alpha\beta$ M q	$r_i = \frac{\alpha\beta N_0 q f_i}{(M+qf_i+\psi)} e^{-\left[iM+q\sum_{j=1}^{i} f_j + i\psi\right]}\left[e^{(M+qf_i+\psi)} - 1\right]$

Desired parameters

P	standing stock
T	throughput
q	catchability
Z_c, Z_f	total attrition
F_c, F_f	fishing mortality
M	natural attrition
H_c, H_f	harvest ratio

Nuisance parameters

α	type 1 tag retention and survivorship
β	proportion of recaptured tags that are reported and useful
ψ	type 2 tag slippage and mortality

Input data

r_i	tag returns in time period i
c_i	catch in time period i
f_i	effort in time period i
$\bar{c}$	average catch per time period
$\bar{f}$	average effort per time period
N_0	number of tags released

model reverts to a two-parameter form.

Fitting the Models

Parameters were estimated by fitting the models given in Table 1 to the tag return results, with input of catch or effort data and input of independent estimates of $\alpha\beta N_0$ and ψ. Fitting was conducted iteratively with either the generalized Marquardt algorithm (Conway et al. 1970) or the simplex algorithm of Nelder and Mead (1965). The varying attrition model could be fitted with all three parameters being adjusted or with one or two of the parameters fixed.

Because finding a tagged fish among many untagged fish is a rare event, we presumed that the statistical distribution of tag counts is approximately the Poisson distribution. We therefore used a square root transformation (Sokal and Rohlf 1969, p. 384).

Goodness-of-fit, G, was measured by the proportion of the total variance in the observed data accounted for by the model, i.e.

$$(8)\quad G = 1 - \frac{S_{\min}}{(n-k)\sigma_T^2}$$

where $S_{\min}$ is the minimum residual sum of squares, n is the number of data points used in the analysis, k is the number of parameters estimated, and σ_T^2 is the variance of the transformed input data.

Confidence Limits

When fitting two-parameter models, the boundary of the joint confidence region of the two parameters corresponds to a contour line on the sum-of-squares surface at which the residual sum of squares is equal to a critical value defined by

$$(9)\quad S_{\text{crit}} = S_{\min}\left[1 + \frac{k}{(n-k)} F_{0.05(k,n-k)}\right]$$

where $F_{0.05(k,n-k)}$ is the critical value of the F-distribution at probability level 0.05 with k and $n-k$ degrees of freedom and where k is the number of data points used in the analysis (Conway et al. 1970). A numerical searching algorithm was devised to trace the contour on the sum-of-squares surface. With the varying attrition model, when three parameters were fitted, it was necessary to trace a critical sum-of-squares shell in three dimensions with the critical sum-of-squares given by Equation 9 with $k = 3$. Confidence intervals for individual parameters were obtained from the extremes of the joint 95% confidence region.

Note that except for the actual observed tag return data, uncertainties in input data are ignored in this method of calculating confidence intervals.

Determining Values for β

Estimating β is complicated by the fact that tagged fish can be found in a variety of ways. For the purpose of this analysis we assumed two discovery modes: by fishermen and by personnel of shore-based processing facilities. Recaptured tags can be broken into usable returns from fishermen, unusable returns from fishermen, tags found by fishermen but not returned, and tags missed by fishermen. The latter category can be further broken into usable returns from shore, unusable returns from shore, tags found ashore but not returned, and tags not found at all. An expression β can be derived as follows. Let u_f, u_s = number of usable returns from fishermen and from shore, v_f, v_s = number of unusable returns from fishermen and from shore, ζ_f = tags returned as a proportion of tags found by fishermen, and ζ_s = tags returned as a proportion of tags missed by fish-

ermen (i.e. as proportion of all tags that came ashore, whether found or not). The β factor is the ratio of the number of usable returns to the total number of recaptures and is given by

$$(10)\quad \beta = \frac{u_f + u_s}{\frac{u_f + v_f}{\zeta_f} + \frac{u_s + v_s}{\zeta_s}}.$$

Assumptions of the Models

In addition to the usual assumptions that tagged and untagged fish are equally vulnerable to fishing gear, a series of assumptions was made in deriving the set of analytical models. Simulations were conducted to investigate the consequences to the parameter estimates of violating some of these assumptions (P. Kleiber, A. W. Argue, J. R. Sibert, and R. Farman, unpubl.).

Temporal distribution of tag releases

One assumption is that all tags are released at time zero rather than throughout the first time interval. This is correct for the aggregate data set but not so for most subarea data. The simulation results showed that the models are insensitive to this problem as long as tag returns are available for more than a few time intervals.

Constant parameter values

A principal assumption is that there is little variation in parameter values during the tagging experiment. To use the catch-based models, the population and the attrition rate should be constant; to use the effort-based models, the catchability and the attrition rate should be constant. A subsidiary assumption, for all but the variable attrition model, is that variations in fishing mortality are small relative to the total attrition rate. Simulation showed that the models are robust to large cyclical variations from steady state. In this case the estimates are close to the average values of the varying parameters. If there are large one-way trends, the models are less robust, and the standing stock and catchability estimates tend to reflect the starting values more than the averages.

A result detrimental to effective fishery management would occur if the harvest ratio was so underestimated that the fishery appeared capable of sustaining increased fishing pressure when in fact it could not. In the simulation exercise, the scenarios under which this could happen involved a drastic downward trend in the population, particularly when this was in response to a sharp decrease in recruitment or a sharp increase in mortality. A sharp decrease in recruitment or increase in mortality by a factor of 2 caused underestimation of the harvest ratio by less than a factor of 1.4. Thus, large departures from steady state cause smaller underestimates of the harvest ratio.

Parameters that apply to individual fish, such as attrition rate, are also assumed to be the same for all fish, though we might expect that such parameters would vary as a function of size or age. In a preliminary analysis we found little evidence for an effect of fish size on total attrition rate (see below).

Territory covered by tagging experiment

An implicit assumption in the derivation of the models is that the stock, of which P is a measure, is a clearly defined entity. However, the effective boundary of the stock which the tagged fish represent is not so clearly defined when the area of operation of a fishery is surrounded by unfished areas and when the fish in the fished area can exchange with fish in the unfished area. In such a case, the territory occupied by a cohort of tagged fish can be expected to expand with time. However, the number of tagged fish simultaneously diminishes due to attrition, which thereby limits the duration of the experiment. The effective size of the territory covered by the tagging experiment therefore depends on the relative rates of migratory expansion and attrition. A simulation of this situation resulted in an estimated stock size corresponding to the population occupying a zone approximately twice the area of the fished zone.

Data Used in Analyses

Table 2 gives the tag return data and other input data used in the analyses. Two data sets were organized differently from the others. These are the data for the whole study area, i.e. aggregate data, and for the Trust Territory and Guam subarea. For these we combined the returns from many tag release episodes according to time at large (elapsed time between release and recapture) without regard to the actual dates of release and recapture. Thus the returns in any months-at-large category were not necessarily all contemporaneous. We did this because detailed catch and effort statistics were not available for all of the release episodes which occurred at many times throughout the 3 yr of the release phase of the tagging experiment and at many places throughout these large areas. This lack of fishery statistics precluded analyzing the release episodes separately. No fundamental change to the models was necessary except that a constant average catch rate had to be assumed because of lack of better data and because the tags, being noncontemporaneous, could not all be associated with the same historical series of catch rates.

The tag data for all subareas other than the Trust Territory and Guam refer to releases and recaptures during specific date spans, 10-d periods for New Zealand and months for other areas.

In Table 2, the t columns identify either the months-at-large category or the specific date span of recapture. The r_i columns give the number of usable recaptures in the territory in question. The few recaptures by the tagging vessel were excluded for all data sets because the fishing effort of this vessel was, for the most part, identical to the places and times of tag release. Returns with unknown dates of recapture were also excluded. If an imperfectly known recapture date could be ascertained to fall within a range of dates such that the extent of the range was less than half the time from release to the midpoint of the recapture range, then the return was accepted and the recapture date taken to be the midpoint of the range. Otherwise the return was rejected. For some individual subareas, the returns were additionally filtered (see below).

The c_i columns in Table 2 give the tons of skipjack caught, and the f_i columns, if present, give the effort in boat days, or purse-seine sets in the case of New Zealand. Except for the Trust Territory and Guam, catch and effort for the first recapture period in each subarea data set were prorated to adjust for timing of tag release during the initial period.

Average monthly catch, $\bar{c}$, and effort, $\bar{f}$, were used in some models. The averages were calculated over the period of time included in the data set. Months with zero catch and effort were included in the average. Catch and effort in individual months could be considerably different from the average, particularly for the highly seasonal New Zealand fishery. Catch and effort were averaged for December–March in New Zealand.

In several cases, the first one or two recapture periods in a

TABLE 2. Tag return and other data used to estimate population parameters. Includes aggregate and subarea da

Aggregate data $\alpha\beta = 0.60$ $\bar{c} = 19000$			Trust Territory and Guam $\alpha\beta = 0.68$ $\bar{c} = 4900$			New Zealand $\alpha\beta = 0.37$ $\bar{c} = 2300$ $\bar{f} = 150$				Papua New Guinea $\alpha\beta = 0.68$ $\bar{c} = 2000$ $\bar{f} = 670$				
t	r_t	c_t	t	r_t	c_t	t	r_t	c_t	f_t	t	r_t	c_t	f_t	
$N_a = 140000$			$N_a = 8887$			$N_a = 2678$				$N_a = 6009$				
a1	2820	19000	1	45	4900	790221	2	336	37	a7905	20	379	114	7
2	506	19000	2	14	4900	790301	223	2396	120	a7906	391	2153	598	7
3	379	19000	3	10	4900	790311	7	148	18	7907	208	2972	1035	7
4	261	19000	4	43	4900	790321	30	215	24	7908	100	2492	913	7
5	198	19000	5	35	4900	790401	1	3	1	7909	58	1840	899	7
6	120	19000	6	16	4900	791121	0	25	7	7910	27	654	614	7
7	115	19000	7	14	4900	791201	0	15	7	7911	5	811	706	7
8	93	19000	8	16	4900	791221	0	35	6	7912	3	286	212	7
9	94	19000	9	5	4900	800101	5	1319	94	8003	1	609	440	7
10	114	19000	10	2	4900	800111	0	2100	87	8004	5	1956	802	7
11	142	19000	11	0	4900	800121	1	1186	86	8005	1	2243	985	7
12	106	19000	12	4	4900	800201	3	1986	135	8006	2	2860	1005	7
13	72	19000	13	0	4900	800211	1	142	18	8007	3	2878	1068	7
14	62	19000	14	0	4900	800221	0	219	33	8008	7	5514	1100	7
15	75	19000	15	3	4900					8009	5	3982	988	7
16	42	19000	16	3	4900	$N_a = 6298$				8010	0	3697	850	7
17	17	19000	17	1	4900					8011	0	3055	831	
18	24	19000	18	1	4900	790301	280	1917	96	8012	0	1800	553	
19	16	19000	19	2	4900	790311	8	148	18	8101	0	222	99	
20	23	19000	20	2	4900	790321	48	215	24	8103	0	1814	447	
21	13	19000	21	0	4900	790401	0	3	1	8104	0	4675	918	
22	8	19000	22	0	4900	791121	0	25	7	8105	0	3085	977	
23	10	19000	23	0	4900	791201	0	15	7	8106	0	3340	962	—
24	11	19000	24	0	4900	791221	0	35	6	8107	1	3421	1077	
25	3	19000				800101	5	1319	94	8108	1	2100	964	—
26	6	19000				800111	3	2100	87	8109	0	1660	805	
27	8	19000				800121	6	1186	86	8110	0	1435	626	
28	7	19000				800201	6	1986	135	8111	0	426	185	8
29	2	19000				800211	1	142	18	8112	0	77	42	8
30	0	19000				800221	0	219	33					8
31	1	19000				800301	1	1191	76					8
32	1	19000				800311	0	493	36					8
33	1	19000				800321	1	113	19					8
34	1	19000				800501	0	18	5					8
35	0	19000				800521	0	49	5					8
36	1	19000				800601	2	35	9					8
37	0	19000				800611	0	12	2					8
38	0	19000												8
39	0	19000												8
40	0	19000												8
41	0	19000												8
														8
														8
														8

subarea data sets.

Solomon Islands (1977) $\alpha\beta = 0.64$ $\bar{c} = 1200$ $\bar{f} = 340$					Fiji $\alpha\beta = 0.80$ $\bar{c} = 350$ $\bar{f} = 120$				Society Islands French Polynesia $\alpha\beta = 0.82$ $\bar{c} = 100$			Gilbert Group Kiribati $\alpha\beta = 0.82$ $\bar{c} = 20$		
f_i	t	r_i	c_i	f_i	t	r_i	c_i	f_i	t	r_i	c_i	t	r_i	c_i
	$N_o = 1709$				$N_o = 11\,646$				$N_o = 823$			$N_o = 4403$		
114	7711	17	754	317	a8004	594	185	76	7812	0	60	7807	26	22.6
598	7712	10	365	397	a8005	183	65	86	7901	1	56	7808	159	49.5
1035	7801	0	15	73	8006	6	76	42	7902	4	64	7809	82	63.5
913	7803	0	15	34	8007	11	116	33	7903	4	91	7810	78	76.3
899	7804	3	941	460	8008	19	90	49	7904	1	110	7902	0	0.8
614	7805	5	888	465	8009	2	99	20	7905	3	170	7903	0	3.8
706	7806	1	1526	515	8010	2	79	21	7906	2	130	7904	1	9.4
212	7807	5	1973	551	8011	2	106	45	7907	0	94	7905	0	8.3
440	7808	3	1587	512	8012	11	229	41	7908	0	89	7906	0	26.8
802	7809	4	2304	517	8101	27	737	198	7909	0	48	7907	0	8.8
985	7810	7	2317	497	8102	42	862	161	7910	0	62	7908	0	12.1
1005	7811	5	2915	514	8103	35	953	193	7911	0	100	7909	0	6.8
1068	7812	5	2723	518	8104	8	605	150						
1100	7901	1	1106	223	8105	12	536	138	$N_o = 896$					
988	7904	2	1438	305	8106	10	418	132						
850	7905	0	1788	485	8107	5	387	140	a7901	0	7			
831					8108	1	76	23	7902	2	64			
553	Solomon Islands (1980) $\alpha\beta = 0.54$ $\bar{c} = 2200$ $\bar{f} = 490$				8110	3	77	33	7903	1	91			
99					8111	1	143	82	7904	0	110			
447					8112	2	298	147	7905	0	170			
918					8201	0	600	224	7906	0	130			
977					8202	1	686	226	7907	0	94			
962					8203	1	696	241	7908	0	89			
1077	t	r_i	c_i	f_i	8204	0	485	231	7909	0	48			
964					8205	0	589	275	7910	0	62			
805	$N_o = 2012$				8206	0	285	168	7911	0	100			
626					8207	0	118	81	7912	1	54			
185	8006	9	354	179	8208	0	42	31	8001	0	190			
42	8007	28	2550	558					8002	0	130			
	8008	25	2778	554					8003	0	83			
	8009	9	2770	574					8004	0	82			
	8010	8	3244	566					8005	0	160			
	8011	20	3313	566					8006	0	97			
	8012	16	2774	594					8007	1	78			
	8101	18	1531	463					8008	0	32			
	8104	5	1210	258					8009	0	55			
	8105	5	1881	560					8010	0	150			
	8106	11	2934	614					8011	0	130			
	8107	4	2796	628					8012	0	180			
	8108	6	3474	640										
	8109	2	2631	639										
	8110	0	2087	630										
	8111	0	2131	632										
	8112	1	1231	415										

data set were disregarded in the analysis. In Table 2 the rows corresponding to these are preceded by an "a." The early returns in any tagging experiment can easily be anomalous because of inadequate mixing of tagged fish in the tagged population. In the present analysis, early returns were disregarded if there was good reason to assume a problem with mixing in the first recapture period(s), and if doing so significantly improved the ability of the model to fit the data.

For each subarea other than the Trust Territory and Guam, releases and recoveries were selected to make the analysis relevant to a local fishery. Releases made outside the area of the local fishery were eliminated as was one release of fish of a substantially different size range than were caught in the local fishery. Tags recovered outside the subarea of release were not considered. Details of tag selection are presented in individual reports prepared for each subarea (Kearney 1982a; Argue and Kearney 1982, 1983; Gillett and Kearney 1983; Kleiber and Kearney 1983; Tuna Programme 1984a, 1984b).

Results and Discussion

Estimation of Nuisance Parameters

Considerable effort was expended to maintain high standards in the tagging procedure (Kearney and Gillett 1982) to maximize α, in the tag return system (Kearney 1982b) to maximize β, and in the quality of the tags themselves to minimize ψ. To analyze the results, however, it was necessary to estimate values for these nuisance parameters.

Estimates of $\alpha\beta$ *with the variable attrition model*

The variable attrition model (last equation in Table 1) was fitted to the five data sets containing effort in Table 2. N_o was set to the values given in Table 2, and the parameters M, q, and $\alpha\beta$ were adjusted by the fitting procedure. In three cases the process converged to impossible values (negative M or $\alpha\beta$ greater than 1), and in two cases possible values resulted. Investigation of the three-dimensional confidence regions for the latter two cases (Fig. 2 and 3) revealed that $\alpha\beta$ was very ill-defined by the analysis. The 95% confidence range (approximately 0.05−1.0) covers most of the possible range (0−1).

Estimation of β

Estimation of β using Equation 10 requires the factors ζ_f and ζ_s. A range of values for ζ_f can be obtained from the double tagging results in Fiji. Using the approach of Bayliff and Mobrand (1972), an estimate of 0.997 (95% confidence range 0.82−1.0) was obtained for the quantity $\rho d_f \zeta_f$, where ρ is the short-term (type 1) tag retention (1 minus tag shedding rate) and where d_f is the proportion of recaptured tags that are discovered by fishermen (Tuna Programme, unpubl. analyses). This range also applies to ρ, d_f, and ζ_f individually because all three quantities can only be in the range (0−1).

In a tag-plant experiment designed to measure ζ_s, 25% of the planted tags were returned, all from shore-based processing facilities, principally in Pago Pago. This experiment was conducted more than 1 yr after most of the recoveries from the regular tagging program were obtained from shore facilities. Thus, it is possible that the low recovery of planted tags reflects a more recent problem in the tag recovery system, or a problem specific to seine-caught fish from New Zealand processed in Pago Pago. Unfortunately, tag-plant experiments were not done on pole-and-line caught fish or on fish destined for other processing facilities. It is also possible that tags placed in dead

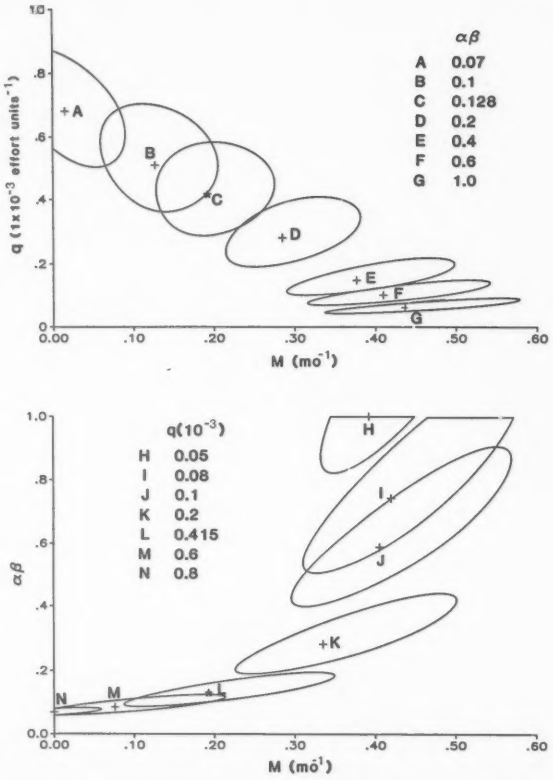

FIG. 2. Confidence regions for estimates of the three parameters of the variable attrition model using the Papua New Guinea data set. In the upper plot, slices through the confidence region at various levels of $\alpha\beta$ are shown. The $\alpha\beta$ axis extends downward from the plane of the page. In the lower plot the figure is rotated forward about the M-axis so that the q-axis rises upward from the plane of the page. Slices at various levels of q are shown. In the upper plot, the crosses give the best-fitting q and M values with $\alpha\beta$ fixed at each level, and in the lower plot the crosses give the best-fitting $\alpha\beta$ and M values with q fixed at each level. The star in each plot gives the best-fitting point for the three-parameter fit.

fish are more easily lost than tags placed in live fish. Taking the tag-plant results at face value, ζ_s could have been as low as 0.25. However, ζ_s might very well have been higher for Pago Pago and other processing facilities during the time that tagged fish from live releases were passing through these facilities. Worst and best case values of 0.25 and 1.0 were assumed for ζ_s.

Assuming the ranges given above for ζ_f and ζ_s, worst and best case values of β were calculated by Equation 10 and are given in Table 3.

Estimation of α

The parameter α depends on type 1 mortality and type 1 tag shedding. As shown above, the type 1 tag shedding rate, $1 - \rho$, must be low. Type 1 tagging mortality is more difficult to determine. However, high tag return rates (>50%) have been observed in the eastern Pacific (Anonymous 1978). This strongly suggests that the combination of type 1 tagging mortality and tag shedding was low. This conclusion is further

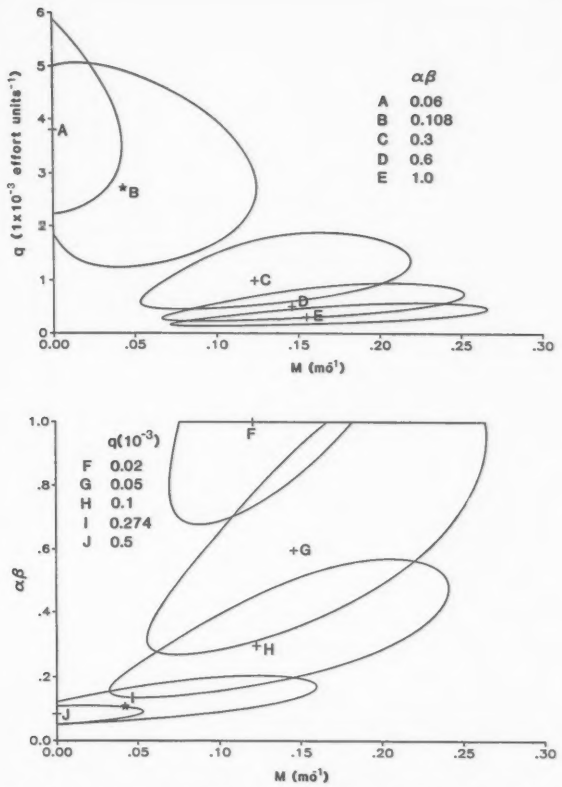

FIG. 3. Confidence regions for estimates of the three parameters of the variable attrition model using the 1980 Solomon Islands data set. In other respects, this figure is similar to Fig. 2.

supported by an experiment with captive skipjack in which no significant difference in mortality between tagged and untagged fish was observed (R. E. Kearney unpubl.).

In the absence of further quantitative information, a figure of 10% has been assumed here for the type 1 losses, i.e. a value of 0.9 for α.

Values of $\alpha\beta$ used in subsequent analyses

The values of $\alpha\beta$ used as input to the analytical model are given at the head of each data set in Table 2. These were derived from an assumed value of 0.9 for α and a value for β midway between the worst and best case values given in Table 3.

Type 2 tag loss, ψ

The parameter ψ can include type 2 shedding and also type 2 mortality. Type 2 shedding estimated from double tagging was $0.0073 \cdot mo^{-1}$ (Skipjack Programme 1981). Type 2 mortality is not so readily estimated, but the double tagging results suggest that it is a minor factor. Except for fish smaller than 45 cm, the proportion returned of the double-tagged skipjack was not significantly less than that of single-tagged skipjack released at the same time (Skipjack Programme 1981). The difference noted for small fish could have resulted from type 1 (affecting α) or type 2 (affecting ψ) losses. We looked for and did not find any influence of size on total attrition, $Z + \psi$ (see next section), as would be expected if there was significant, size-dependent, type 2 mortality. This suggests that the reduction in returns for the small double-tagged fish was predominantly a type 1 phenomenon. In any case, these small fish accounted for less than 15% of the returns considered in this report. Type 2 mortality was therefore assumed to be zero, and the value of ψ for all data sets was taken to be the estimate of type 2 tag shedding.

TABLE 3. Worst and best case values of β are calculated from Equation 10 using worst and best case assumptions about ζ (0.82 and 1.0 for ζ_f; 0.25 and 1.0 for ζ_s).

Data set	Where found	Tag returns: Useable u	Tag returns: Reject v	β: Worst case	β: Best case
Aggregate	Fishermen	4641	125	0.47	0.87
	Shore	711	706		
Trust Territory and Guam	Fishermen	190	9	0.58	0.94
	Shore	26	6		
New Zealand	Fishermen	231	12	0.19	0.64
	Shore	403	352		
Papua New Guinea	Fishermen	838	7	0.62	0.90
	Shore	0	82		
Solomon Islands (1977)	Fishermen	65	1	0.55	0.88
	Shore	3	8		
Solomon Islands (1980)	Fishermen	167	6	0.43	0.77
	Shore	0	45		
Fiji	Fishermen	977	23	0.80	0.98
	Shore	1	0		
Society Islands	Fishermen	20	0	0.82	1.0
	Shore	0	0		
Gilbert Group	Fishermen	346	0	0.82	1.0
	Shore	0	0		

TABLE 4. Distribution of tag returns among subareas for three classes of size at release. The total returns are not necessarily the same as the totals of returns in Table 2 because in this case, tags were selected with regard to the existence of accurate length measurement at release and accurate time of recapture, but without regard to other selection criteria involved in assembling the data sets in Table 2.

Data set	Fork length <45 cm	Fork length 45–55 cm	Fork length >55 cm	Total tag returns
Aggregate	634	3536	630	4800
Fiji	139	1380	114	1633
Solomon Islands	157	290	55	502
Papua New Guinea	14	573	311	898
Palau	204	52	51	307
Ponape	1	95	44	140
New Zealand	100	536	8	644
Wallis and Futuna	3	67	6	76
Tuvalu	1	21	6	28
Gilbert Group	3	354	8	365

Effect of Size at Release on Attrition

To test for the effect of size at release on attrition rates, the

TABLE 5. Estimates of tag attrition (mo^{-1}) with 95% confidence intervals for three classes of size at release. Results are given for the aggregate data set and for subareas with 20 or more returns in more than one size class. One data set, WTG, is the sum of returns for Wallis and Futuna, Tuvalu, and the Gilbert Group.

Data set	Fork length		
	<45 cm	45–55 cm	>55 cm
Aggregate	0.17 (0.15–0.20)	0.17 (0.14–0.20)	0.27 (0.22–0.33)
Fiji	0.18 (0.10–0.32)	0.57 (0.09–0.24)	0.12 (0–0.33)
Solomon Islands	0.17 (0.11–0.26)	0.18 (0.12–0.25)	0.27 (0.17–0.42)
Papua New Guinea		0.34 (0.21–0.58)	0.63 (0.42–0.92)
Palau	0.21 (0.17–0.27)	0.15 (0.08–0.25)	0.37 (0.19–0.69)
Ponape		0.20 (0.13–0.29)	0.22 (0.06–0.48)
New Zealand	1.1 (0.9–10.4)	1.2 (0.9–10.5)	
WTG		0.24 (0.14–0.46)	0.30 (0.07–0.69)

aggregate tagging data and seven subsets of the data were broken into three categories of size at release, <45, 45–55, and >55 cm (Table 4). Six subsets consisted of subarea data with 20 or more returns in at least two of the size categories. One additional subset was made up of returns from three subareas which in combination gave 20 or more returns in two size classes.

The aggregate and six of the subsets were put into months-at-large classes in the same way as the aggregate data in Table 2. The New Zealand subset was broken into 10-d periods. Total attrition was estimated by fitting the first model in Table 1 to the data, using a constant catch rate for subarea as well as aggregate data under the assumption that the effects of changing catch rate on attrition estimates would be roughly equivalent for the different size classes.

The resulting attrition estimates (Table 5) can be used to compare size classes within a subarea or the aggregate data set, but they should not be used to compare attrition rates between countries. The only significant result is a higher attrition rate for large fish in the aggregate case. However, a large portion of the large fish were released in Papua New Guinea (Table 4) where, as we shall see (Table 6), the attrition rate was particularly high. Therefore the result for aggregate data could be a Papua New Guinea effect instead of a size effect, a conclusion that is supported by the lack of significant effect of size for individual subarea data.

Tag Attrition Curves

The decline in tag return rate with time is shown for the aggregate data set in Fig. 4. As indicated in Table 2, the value for the first month was not included in the fitting. The bump in the observed data at approximately 1 yr could be the result of seasonality in the fisheries. Most fisheries in the region have a period of higher fishing effort each year lasting from a little over 1 mo (New Zealand) to several months (Papua New Guinea). Because tags tended to be released in each subarea during these periods, it is to be expected that a surge of tag returns would coincide with increased fishing, approximately 1 yr following tagging.

The predicted values decline smoothly in Fig. 4 because we could not account for variations fishing activity in the aggregate case. When we were able to account for such variations, the predicted values decline jerkily (Fig. 5) because they reflect variations in catch, as well as the steady decline due to all the components of attrition.

Parameter Estimates from the Analytical Model

Table 6 gives goodness-of-fit and parameter estimates obtained from all but the variable attrition model. Table 7 gives the goodness-of-fit and parameter estimates obtained by fitting the variable attrition model to the data sets for which effort data were available.

The results from the variable attrition model are close in most cases to the corresponding results from the fixed attrition models. The goodness-of-fit was not improved, and the catchability estimates are likewise much the same. The last two columns in Table 7 match well except for the New Zealand results. In this case the discrepancy between variable attrition and fixed attrition models may be due to the large degree of seasonality in the New Zealand fishery, the resulting large variation in attrition being more easily accounted for by the possibility of variable attrition in the model.

The parameter estimates in Tables 6 and 7 form the basis of Skipjack Programme reports for individual subareas wherein the implications of these results to fisheries in the subareas are discussed in detail.

Reliability of the parameter estimates

The confidence limits reported in Tables 6 and 7 do not include uncertainty in the values of the nuisance parameters, and therefore, the confidence ranges in the tables are minimum estimates.

Accounting for uncertainty in ψ (the type 2 shedding rate) would directly affect the confidence ranges of attrition, and thus to a similar extent the estimates of throughput and harvest ratio. The 95% confidence range of ψ is $0.0031-0.0116 \cdot mo^{-1}$. However, given its small magnitude relative to the attrition rate, its variance is unlikely to significantly affect the confidence ranges of any of the above parameters.

Accounting for the uncertainty in the value of $\alpha\beta$ would affect the confidence ranges of all parameters except Z_c and Z_f. The range between the best and worst case estimates of β can be large (Table 3), and the assumed value of α is a guess based on little quantitative information. The confidence ranges given in Tables 6 and 7 would be larger if the uncertainty in $\alpha\beta$ had been included, which can be seen in the three-parameter confidence regions for Papua New Guinea and Solomon Islands, 1980 (Fig. 2 and 3). It is interesting to note, however, that the effect of uncertainty in $\alpha\beta$ is dependent on whether this quantity is in the upper or lower part of its possible range (0–1). Thus the confidence range of M is considerably reduced if $\alpha\beta$

TABLE 6. Results from the analytical models in Table 1 using data in Table 2. The upper figu
the 95% confidence limits. Effort data was not available for all data sets; therefore, there are
land and Society Islands, data from releases in consecutive periods were used in one fitting of t
ance explained by model with effort data, P = standing stock (Mt), T = throughput (Mt·
attrition (mo^{-1}) with catch data, Z_f = attrition (mo^{-1}) with effort data, F_c = fishing mortali
vest ratio (dimensionless) with catch data, H_f = harvest ratio (dimensionless) with effort da

Data set	G_c	G_f	P (10^3)	T (10^3)	q (10^{-3})	Z_c
Aggregate	0.95		3000 2500–3700	520 460–590		0.17 0.15–0.20
Trust Territory and Guam	0.71		660 370–1310	150 100–250		0.23 0.14–0.36
New Zealand	0.91	0.85	13 10–17	5.0 3.8–7.0	1.2 0.8–1.7	0.38 0.30–0.52
Papua New Guinea	0.96	0.95	35 27–45	13 11–16	0.090 0.060–0.144	0.38 0.32–0.46
Solomon Islands (1977)	0.69	0.52	49 25–124	11 7–19	0.027 0.008–0.065	0.23 0.13–0.34
Solomon Islands (1980)	0.63	0.68	89 49–185	13 9–22	0.056 0.029–0.096	0.15 0.07–0.26
Fiji	0.80	0.68	39 20–79	7.3 4.8–11.4	0.081 0.036–0.164	0.19 0.13–0.26
Society Islands	0.36		9.7 1.8–67.1	5.7 2.1–20.1		0.59 0.20–1.30
Gilbert Group	0.91		1.0 0.5–2.1	0.38 0.24–0.64		0.37 0.16–0.69

pper figure in each cell is the best parameter estimate, and if given, the lower two figures are there are some blank cells for quantities that depend on the effort value. For the New Zea- tting of the model. G_c = % of variance explained by model with catch data, G_f = % of vari put ($Mt \cdot mo^{-1}$), q = catchability ($sets^{-1}$ in New Zealand, $vessel\text{-}days^{-1}$ elsewhere), Z_c = g mortality (mo^{-1}) with catch data, F_f = fishing mortality (mo^{-1}) with effort data, H_c = har- effort data.

$_c$	Z_f	F_c	F_f	H_c	H_f
17		0.0063		0.037	
-0.20		0.0051–0.0077		0.032–0.042	
23		0.0074		0.032	
-0.36		0.0038–0.0131		0.020–0.048	
38	0.39	0.17	0.18	0.46	0.46
-0.52	0.29–0.62	0.13–0.22	0.12–0.25	0.33–0.60	0.29–0.65
38	0.47	0.058	0.061	0.15	0.13
-0.46	0.35–0.65	0.045–0.075	0.040–0.097	0.13–0.18	0.10–0.16
23	0.14	0.024	0.0092	0.11	0.067
-0.34	0.01–0.29	0.010–0.049	0.0027–0.0223	0.06–0.17	0.034–0.270
15	0.16	0.025	0.027	0.16	0.17
-0.26	0.09–0.26	0.012–0.046	0.014–0.047	0.10–0.25	0.11–0.24
19	0.19	0.0091	0.0097	0.048	0.056
-0.26	0.12–0.26	0.0044–0.0174	0.0043–0.0197	0.031–0.072	0.030–0.084
59		0.010		0.017	
-1.30		0.001–0.055		0.005–0.048	
37		0.019		0.052	
-0.69		0.009–0.038		0.031–0.083	

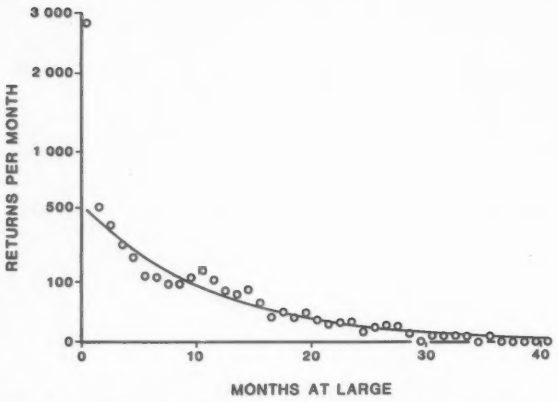

FIG. 4. Aggregate tag attrition curve. Points are the aggregate tag return rates (Table 2). The solid line gives the expected values based on the best fit of first model form in Table 1. The *y*-axis is a square root scale.

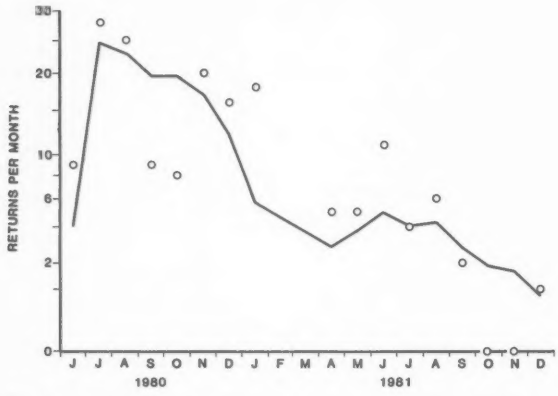

FIG. 5. Example tag attrition curve for a subarea (Solomon Islands, 1980). In other respects, this figure is similar to Fig. 4.

is known to be greater than 0.4, but more precise definition of $\alpha\beta$ within the range 0.4−1 would not narrow the confidence range of M much further (Fig. 2 and 3).

It must be stressed that the parameter values for individual subareas, and overall, apply to the time of the tagging experiment. Since that time, significant changes have occurred in several fisheries in the region. In addition, periodic major environmental events occur that presumably could affect skipjack populations in the tropical central and western Pacific waters (Wyrtki 1975; Donguy and Henin 1978).

Standing stock and throughput

The standing stock in different areas, under conditions of uniform stock density, would be proportional to the size of the area. Therefore, differences among individual country results would reflect the size of the areas covered by the different tagging experiments, which by design roughly covered the area of the locally based fisheries. Such is evident in Table 6 by comparison of P for the Gilbert Group with P for the other individual subareas. The Gilbert Group estimate is smaller than all others, and the "fishery" was a single vessel survey concentrated near a single atoll, a much smaller area than the other individual country fisheries. The aggregate estimate of P is much larger than the sum of estimates for individual subareas in Table 6 because these are only a portion of the area included in the aggregate. Between these extremes, differences among subareas are difficult to interpret, firstly because of the large overlapping confidence intervals and secondly because of the difficulty in evaluating the effective area covered by the fisheries during the tag recovery period.

TABLE 7. Results from varying attrition model with data sets containing effort values in Table 2. The parameter $\alpha\beta$ was fixed to the values given in Table 2. The format of the entries in each cell is the same as in Table 6. For comparing M with results from fixed attrition models, values of attrition minus fishing mortality ($Z_f - F_f$) from Table 6 are included in the last column of this table.

Data set	G	q (10^{-4})	M	$Z_f - F_f$
New Zealand	0.85	13 9−18	0.35 0.25−0.58	0.21
Papua New Guinea	0.95	0.88 0.60−1.30	0.41 0.31−0.55	0.41
Solomon Islands (1977)	0.51	0.26 0.08−0.64	0.13 <0.01−0.27	0.13
Solomon Islands (1980)	0.68	0.55 0.39−0.96	0.14 0.07−0.22	0.13
Fiji	0.68	0.79 0.35−1.56	0.18 0.12−0.24	0.18

Throughput, T, should be only approximately proportional to the size of the fished area, since throughput is the product of attrition and standing stock and attrition has a component due to emigration which is expected to vary inversely with the size of the fished area.

Attrition

Attrition and its components are not expected to be proportional to the area covered by the experiment. However, attrition is not necessarily independent of area because it includes a component due to dispersive movement of fish. This component tends to increase in importance with decreasing size of the area under consideration. Therefore the attrition is expected to vary inversely with area and, for large areas, approach a dispersion-free attrition rate. It is probably for this reason that the aggregate attrition estimate was lower than all but one of the individual country estimates (though only three have non-overlapping confidence intervals).

Under the assumption of steady state, the attrition rate is also the population turnover rate. Simulation modelling showed that in a non-steady-state situation the attrition estimate would tend to reflect the average attrition over the time of the experiment. Thus if the lack of steady state is attributable to seasonal fluctuations, and tags are returned over a period of at least 1 yr, then the attrition estimate would reflect the yearly average population turnover. Furthermore, simulation showed that in nonequilibrium conditions (i.e. when the sum of inputs is different from the sum of outputs), the estimate of Z_c tends to be closer to the sum of inputs and Z_f closer to the sum of outputs. The implication is that if Z_c is larger than Z_f, then the population is increasing whereas if Z_c is less than Z_f, then the population is decreasing. The only cases in which there were

appreciable differences between Z_c and Z_f were the results from the 1977 Solomon Islands data set ($Z_c > Z_f$) and from the Papua New Guinea data set ($Z_c < Z_f$). (The confidence regions given in Table 6 are not relevant in judging the significance of a difference between estimates of Z_c and Z_f when these parameters are obtained from the same data sets because there is likely to be a high positive covariance between the two estimates, which would tend to minimize the variance of the difference between them.) It may only be fortuitous that the increasing trend in P in Solomon Islands (October 1977 versus June 1980) was consistent with that predicted by the 1977 estimates of Z_c and Z_f, since the confidence intervals for the two estimates of P are large and overlapping. (In this case, where the results from two independent sets are compared, the confidence regions in Table 6 are relevant.) The trend predicted for Papua New Guinea could not be checked because there was no further tagging experiment in these waters.

The aggregate estimate of attrition is $0.17 \cdot mo^{-1}$ (0.15–0.20). When fishing mortality is subtracted the remaining attrition is $0.16 \cdot mo^{-1}$. Joseph and Calkins (1969) reported a comparable estimate of skipjack attrition, excluding F, of $0.14 \cdot mo^{-1}$ from a tagging experiment in the northern zone of the eastern Pacific fishery. Ssentongo and Larkin (1973) gave a method for calculating attrition in exploited fish populations given the length of fish at recruitment, the mean length in the catch, and values for the parameters L_∞ and K of the von Bertalanffy growth model. For skipjack, assuming a length at recruitment of 38 cm and a mean length in the catch of 50.4 cm (the mean length of skipjack tagged by Skipjack Programme), and using values of $L_\infty = 62.5$ cm and $K = 0.17 \cdot mo^{-1}$ (Sibert et al. 1983), the predicted value of attrition is $0.24 \cdot mo^{-1}$, which drops to $0.23 \cdot mo^{-1}$ when our estimate of F is subtracted. Pauly (1979) reported a regression equation for predicting natural mortality of a fish species given its von Bertalanffy parameter values and its mean environmental temperature. The regression equation was based on reported natural mortality estimates from a wide variety of fish families (including skipjack among several examples of scombrids). It is unclear to what extent attrition mechanisms other than natural mortality and fishing mortality are included the estimates used to derive the regression equation. Assuming the values given above for L_∞ and K and a mean water temperature of 25°C, the Pauly estimate of natural mortality for skipjack is $0.18 \cdot mo^{-1}$, which is similar to our overall estimate of attrition.

Catchability

Catchability coefficients, q, for pole-and-line gear (Table 6) range from $2.7 \times 10^{-5} \cdot$ fishing day^{-1} for November 1977 tagging in Solomon Islands to $9.0 \times 10^{-5} \cdot$ fishing day^{-1} for May–June 1979 tagging in Papua New Guinea; however, all estimates have overlapping confidence intervals and little can be made of the differences among subareas. For purse-seiners in New Zealand, q is $1.2 \times 10^{-3} \cdot set^{-1}$ or $1.8 \times 10^{-3} \cdot$ fishing day^{-1} using the average of 1.5 sets · fishing day^{-1} for the 1979–80 and 1980–81 New Zealand fishing seasons (Argue and Kearney 1983). This catchability for purse-seiners is 28 times higher than the average for pole-and-line gear in Table 6, which probably reflects greater fishing power for purse-seiners and greater skipjack vulnerability in the coastal waters of New Zealand.

Harvest ratio

Having defined the harvest ratio and having obtained estimates thereof, it is useful to have a bench mark to show whether a given estimate is high, indicating heavy fishing pressure, or low, indicating the possibility for increased yield. The harvest ratio is analogous to the X-factor of Gulland (1971), defined such that

$$(11) \quad Y = XT_v$$

where Y is the potential yield and T_v is the virgin turnover. On the basis of two arguments, Gulland suggested that the maximum yield from a fishery is obtained with a value of approximately 0.5 for X. One argument is based on the Schaefer model and has been shown by Francis (1974) to be unreliable. The other argument is based on the Beverton–Holt yield per recruit model wherein for a broad range of conditions, the maximum yield per recruit is obtained with a value close to 0.5 for X. Beddington and Cooke (1983) argued that for most realistic sets of parameter values, a value of X somewhat smaller than 0.5 gives maximum yield. However, if the Beverton–Holt yield is calculated for a skipjack-like fish with the values of L_∞ and K assumed above and a natural mortality of $0.16 \cdot mo^{-1}$, the harvest ratio (or X) producing maximum yield is seen to be in the neighborhood of 0.5–0.7 with a size at recruitment between 36 and 40 cm (Fig. 6). It should be noted that the sustainability of yields under Gulland's second argument depends on an assumption of constant recruitment, regardless of standing stock level (Beddington and Cooke 1983). Nevertheless, a harvest ratio close to 0.5 would seem to be a good signpost for a skipjack fishery approaching full exploitation.

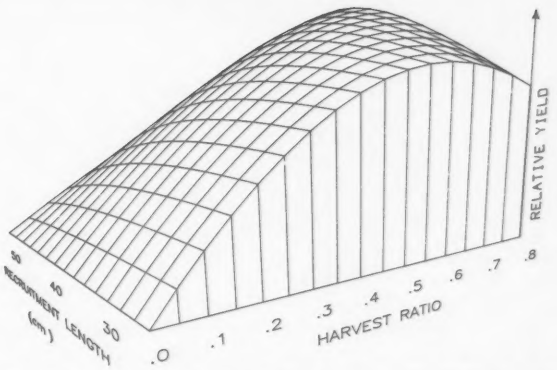

FIG. 6. Beverton-Holt yield surface. Relative yield is plotted as a function of harvest ratio and length at recruitment. Natural mortality is assumed to be $0.16 \cdot mo^{-1}$. L_∞ and K of the von Bertalanffy growth model are assumed to be 62.5 cm and $0.17 \cdot mo^{-1}$, respectively (Sibert et al. 1983).

The estimates of harvest ratio (Table 6) tend to be lower than 0.5. For the aggregate case, the harvest ratio is low, 0.04, implying that fishing is having little impact on the skipjack resource in the study area as a whole. For subareas with well-established commercial fisheries (New Zealand, Papua New Guinea, and Solomon Islands), harvest ratios are higher, 0.15–0.46, while the other subareas, which have small or fledgling fisheries, have low harvest ratios, <0.1. Low harvest ratios for a large part of the study area imply that there is a potential for greatly increased skipjack yield, both within individual subareas and in the study area as a whole. However, recently expanded purse-seine fisheries in the vicinity of the Trust Territory, Papua New Guinea, and Solomon Islands have undoubtedly realized some of this potential.

Conclusion

The results of the analyses given in this paper provide evidence that the resource of skipjack in the study area of the Skipjack Programme is large, its rate of turnover is high, and the rate of mortality due to fishing during the study period was only a small fraction, <0.05, of the rate of turnover. This implies that skipjack catches over the whole study area could be substantially increased from those of the study period. The tag recapture and attrition models used to obtain aggregate estimates and confidence intervals for standing stock, turnover, and fishing mortality were applied to tagging data from subareas with skipjack fisheries for which catch statistics were available. Parameter estimates so derived suggest that the impact of fishing in the smaller areas is larger than the overall impact of fishing.

It should be emphasized that the results reported here apply to the time of the tagging experiment. Substantial development of purse-seining has occurred in the region since that time. This large increase in fishing effort, the large confidence intervals of parameter estimates, and the occurrence of high harvest ratios in some subareas all argue for a cautious approach in planning further development of skipjack fisheries in the central and western Pacific.

The analytical techniques used in this study are based on a set of models derived to address the situation of skipjack and of the tagging experiment conducted by the Skipjack Programme. With due attention to our discussion of assumptions, these techniques could be applied to other situations with similar salient features. The activity of the fishery can vary during the experiment, but large trends in conditions of fishery or environment during the experiment should be a warning signal. The behavior of the fish should be similar across all sizes (ages) that are tagged and should not have large trends during the experiment. These analyses should be used cautiously with long-lived fish that are exposed to the fishery for a long time, but fish like skipjack with a high turnover rate, and consequent short time in the fishery, would be good candidates.

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Microbial Activity and Ultrastructure of Mineral-Based Marine Snow from Howe Sound, British Columbia[1]

Penny S. Amy[2] and Bruce A. Caldwell

Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA

A. H. Soeldner

Electron Microscope Facility, Oregon State University, Corvallis, OR 97331, USA

Richard Y. Morita

Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA

and L. J. Albright

Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6

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Electron microscopy and biochemical measurements of heterotrophic and enzymatic activities were made on marine snow and underlying sediments collected from Howe Sound, British Columbia. SEM showed the marine snow to be accretions of comminuted phytoplankton, fecal pellets, cellular material, and mineral particles bonded by films and coatings of an amorphous material. The amount of amorphous substance decreased as Howe Sound became increasingly marine. Rod, cocoidal, and filamentous bacteria were cultured from the marine snow. Heterotrophic microbial activity increased seaward and was found to be higher in marine snow than in underlying sediments. Phosphatase, protease, laminarinase, and xylanase were also more active in marine snow whereas sulfatase activity was found to be higher in the sediments. β-Glucosidase activity was demonstrated in marine snow only at sampling stations isolated from outer Howe Sound and the Strait of Georgia by a submarine sill.

Des débris marins en suspension et des sédiments sous-jacents prélevés dans le détroit Howe, en Colombie-Britannique, ont été examinés par microscopie électronique et ont fait l'objet d'une mesure biochimique de leurs activités hétérotrophes et enzymatiques. La microscopie a montré que les débris étaient constitués d'accrétions de fins débris de phytoplancton, de pastilles fécales, de matériaux cellulaires et de particules minérales liés par des films et des revêtements de matière amorphe. La quantité de substance amorphe diminuait à mesure que l'on se dirigeait vers la mer. Des bactéries en forme de bâtonnets, de coques ou de filaments provenant des débris en suspension ont été cultivées. L'activité microbienne hétérotrophe augmentait en direction de la mer et était plus importante dans les débris marins que dans les sédiments sous-jacents. La phosphatase, la protéase, la laminarinase et la xylanase des débris marins étaient aussi plus actives tandis que l'inverse était vrai pour la sulfatase. L'activité de la β-glucosidase n'a pu être démontrée que dans les débris marins provenant des stations de prélèvement isolées de la partie externe du détroit Howe et du détroit de Géorgie par un seuil sous-marin.

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Particulate matter in seawater is derived from a number of sources (Riley 1970; Cauwet 1978; Syvitski et al. 1985). The components comprising these particulates include minerals, fragments of phyto-zooplankton, fecal pellets, and particulate organic material formed from previously dissolved organic matter. Collectively, this material has traditionally been called marine snow (due to its appearance in deep green water), "floc", particulate matter, and several other terms. Syvitski and Murray (1981) used the term "agglomerate" to describe organic and inorganic matter weakly held together by ionic charge, surface tension, and organic cohesion. Bacteria can perform an important role in the formation and growth of marine particulates by both the recovery and conversion of dissolved organic matter into particulate form (Paerl 1978) and aggregating other particles together with secretion (Paerl 1973; Syvitski et al. 1985).

Research in the warm waters off the California coast (Alldredge 1976, 1979; Silver et al. 1978; Trent et al. 1978; Shanks and Trent 1979; Alldredge and Cox 1982; Knauer et al.

[1]Technical Paper No. 7925, Oregon Agricultural Experiment Station.

[2]Present address: Biological Sciences, University of Nevada at Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154, USA.

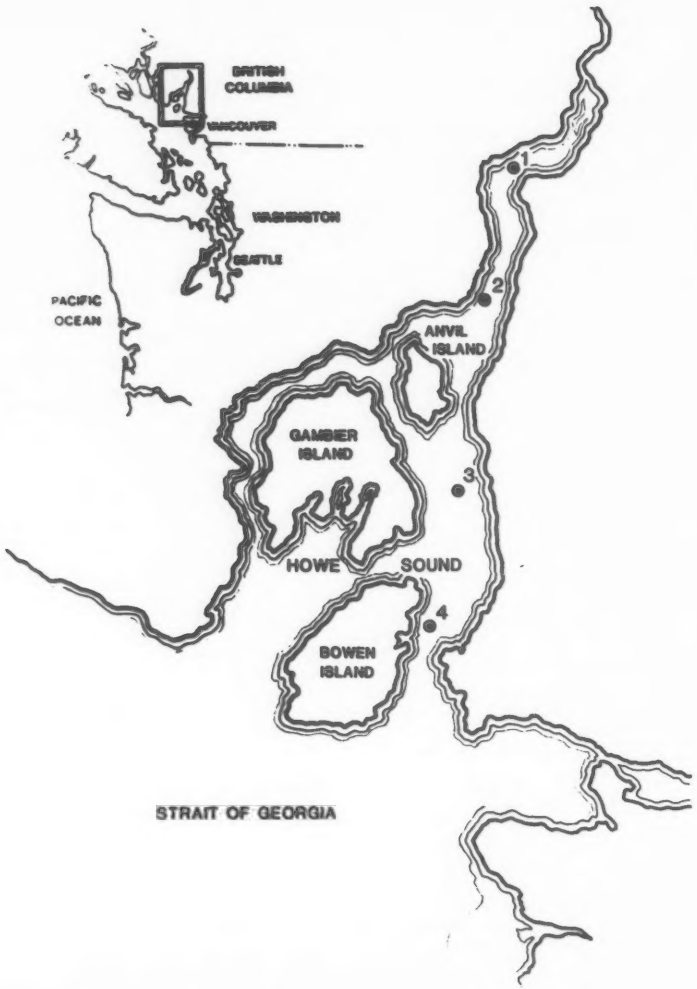

FIG. 1. Study area and sampling stations in Howe Sound, B.C.

1982; Prezelin and Alldredge 1983) and the Sargasso Sea (Riley et al. 1965; Caron et al. 1982) demonstrated the presence of a biologically based marine snow. Syvitski and Murray (1981), working in the cold waters of British Columbia, observed the presence of a second type of agglomerate which is mineral based and contains little biological material. This second type of marine agglomerate is the subject of this research. It is heavily laden with silt and clay from glacial runoff of rivers that discharge into fjords on the northwestern coast of Canada. Both types of agglomerate material may act as sites of nutrient enrichment and regeneration, contain high concentrations of bacteria/phytoplankton available for grazing by zooplankton, generate microscale habitat heterogeneity, and transport organic matter to the sediment at velocities different from individual planktonic organisms.

Little information has been available on the microbial productivity, heterotrophic activity, or enzymatic activities which are probably responsible for significant alterations in the structure and nutritive value of marine agglomerates. Because of the usual loss of nutrient-rich material from the photic zone toward depth in a water column, mechanisms to produce agglomerates which become increasingly richer in microbial cell mass are of interest to the total nutrient budget of benthic and water column organisms.

In this study, we have concentrated on the microbial activity, both by whole organisms in communities and by the enzymes that those organisms produce, of macroagglomerates in the water column and uppermost sediment layer of a Canadian fjord. In addition, we have evaluated the microstructure of the habitat available within marine snow macroagglomerates by scanning electron microscopy (SEM).

Materials and Methods

Sampling Site

Samples for this study were collected in April 1984 from Howe Sound, British Columbia, using the research submers-

ible *PISCES IV*. Howe Sound, located 20 km north–northwest from Victoria, B.C., is the fjord of the Squamish River drainage (~3636 km^2) from the Pacific Ranges of the Coast Mountains (Fig. 1). The 42-km-long fjord broadens from approximately 2 km at the Squamish River delta to 18 km at its terminus in the Strait of Georgia and has a mean water maximum depth of 325 m. The Squamish River is the source of most freshwater and terrestrially derived suspended materials discharged into Howe Sound.

The water masses of Howe Sound and the Strait of Georgia are partially separated by a submarine sill located across much of the mouth of the fjord (outer sill). A second sill (inner sill) crosses the fjord 25 km into the Sound, about 1.5 km north of Anvil Island, at a mean water depth between 35 and 70 m.

Samples were taken at the vicinity of 49°35′N, 123°14′W (station 1), 49°35′N, 123°15′W (station 2), 49°28.5′N, 123°16′W (station 3), and 49°23.5′N, 123°14′W (station 4), with dive sites fixed by bathymetry and by radar and visual triangulation. The four stations comprised a north–south transect along the middle of the main fjord channel. Two stations (1 and 2) were located north of the inner sill and two stations (3 and 4) were located between the outer and inner sills.

Comparison sampling was also done during dives made in Howe Sound and Bute Inlet (October 1983), in Saanich Inlet (March and April 1984), and in Howe Sound (October 1984).

Sampling Methods

Observations were made and samples collected using the manned research submersible *PISCES IV* operated from its support vessle, M.V. *PANDORA II*. Three sampling methods were used with *PISCES IV*.

One method used three butterfly samplers (Niskin) fixed to the submersible's crash bar and triggered by the hydraulic arm. These were used to obtain water samples from desired depths and locations.

Method 2 used an electrically operated water pump to draw samples through a flexible 8.0-cm-diameter plastic tube. The tube inlet, fastened to *PISCES IV*'s hydraulic arm, allowed specific agglomerates or locations (e.g. nephloid layer, layer of dense snow, single large agglomerates) to be sampled. Collections were pumped into numbered acrylic jars mounted by a rotating plate to sequentially position each jar at the pump's discharge port. A brushed nylon cloth filter supported by screening placed over the pressure relief outlet for each jar served to retain the collected material. By maintaining a desired depth, and pumping for several minutes, large quantities of marine snow could be accumulated by this mechanism. Samples were kept in the original water taken at depth after return to the surface. Material from cloth filters was resuspended in small volumes of seawater taken from the acrylic jars. The original structure of the flocculant material was disrupted but maximally recovered by this process. Flocculated material reaggregated with settling, but was resuspended to maintain even distribution for measurements of bacterial counting, heterotrophic glucose utilization, and enzymatic activity. Sediments were diluted 1:10 or 1:100 with filter-sterilized seawater to produce an even slurry for biochemical determinations. Agglomerate and sediment suspensions were then vacuum filtered onto 0.22-μm Millipore filters and air dried to obtain dry weight measurements.

A large rectangular box trap, with top and bottom ends retracted prior to launch, was mounted to the submersible's crash bar and used to recover undisturbed specimens of agglomerates for electron microscopy (EM) and microbial culture. *PISCES IV* could be maneuvered to position the open box to enclose an agglomerate. When the box ends were closed, a water-tight seal was formed which trapped the agglomerates with several litres of seawater.

Following retrieval of *PISCES IV* aboard *PANDORA II*, individual agglomerates captured with the box trap sampler were transferred to Petri dishes containing SLX agar (Amy and Morita 1983) and incubated 10 d at 10°C or prepared for EM.

Electron Microscopy

From the box trap sampler, intact agglomerate samples for EM were floated into glass vials. Seawater was replaced by 3% glutaraldehyde in filter-sterilized buffered seawater and the specimens were fixed for 2–4 h. Samples were transferred to the laboratory in 0.25% glutaraldehyde in filter-sterilized seawater.

For microscopy, samples were placed in dialysis tubing and placed in fresh tap water to remove sea salts and then floated onto 15-mm-diameter cover glasses and freeze dried 24 h at 1×10^{-3} torr. After drying, cover glasses were fastened to aluminum planchets using DUCO adhesive, coated for conductivity with 60:40 Au/Pd alloy by vacuum evaporation, and examined in an AMRAY 1000A scanning electron microscope. Several dozen agglomerates were examined and several hundred SEM images recorded.

Bacterial Counts

Bacterial counts were determined by the acridine orange direct count method (Hobbie et al. 1977; Daley and Hobbie 1975). Bacterial carbon values were determined as described by Albright (1983a, 1983b). Bacteria were released from particles by the pyrophosphate-sonication method of Velji and Albright (1986).

Heterotrophic Activity

Relative heterotrophic uptake of dissolved organic matter was assayed with a modification of the one concentration method of Griffiths et al. (1978). Five millilitres of agglomerate suspension or either a 1:10 or 1:100 sediment dilution (with filter-sterilized seawater) was incubated with 0.39 mCi [^{3}H] glucose (12.6 mCi·mmol^{-1}) for 1 h at in situ temperature. Microbial uptake was terminated by addition of 100 μL of 8% EM-grade glutaraldehyde. The samples were filtered onto 0.45-μm Millipore filters, air dried, and stored in 20-mL scintillation vials for return to Oregon State University. Glucose uptake was quantified using a Beckman LS-8000 liquid scintillation counter with quench correction by the channels ratio method. Uptake was calculated as picomoles of glucose per gram per hour.

Hydrolytic Enzymes

To provide a sufficient concentration of material for analysis, 1–50 cm^3 of agglomerate suspension was concentrated by gentle filtration onto 0.45-μm Millipore filters.

Phosphatase activity was measured using a modification of the spectrophotometric assay of Morita and Howe (1957) and Tabatabai and Bremner (1969). One cubic centimetre of sediment or a filter of concentrated particulate material was

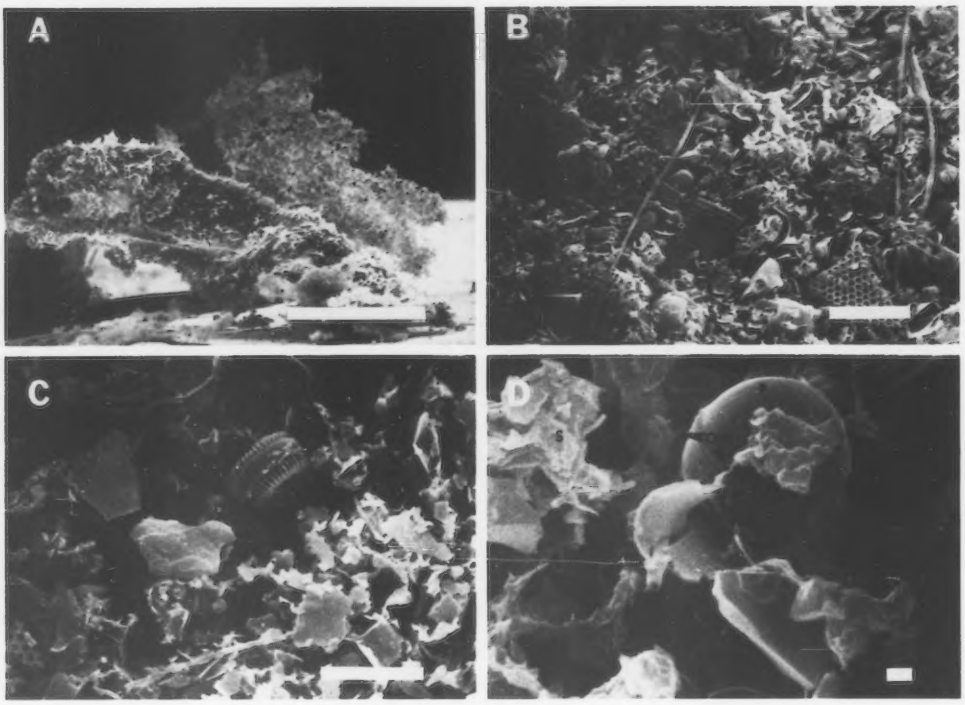

FIG. 2. Marine snow, Howe Sound, station 2, 250 m, October 1983. (A) Typical agglomerate flake structure; bar = 500 μm. (B and C) Agglomerate microstructure characterized by rough texture, great porosity, and much comminution of included particles; bars = 10 μm. (D) Agglomerate components including phytoplankton and planktonic debris (P), triptonic clay and silt particles (S), and amorphous thin films of organic material (OM); bar = 1.0 μm.

brought to a total volume of 2 mL with 0.1 *M* tris(hydroxymethyl)aminomethane (THAM) buffer (pH 7.5), and 1.0 mL of buffer containing 5 m*M* *p*-nitrophenylphosphate was added. After incubation for 1–6 h at 25°C, the reaction was terminated with 2 mL of 0.5 *N* NaOH and 0.5 mL of $CaCl_2$. Following centrifugation for 10 min at 2000 × *g*, the $OD_{410\,nm}$ of the supernatant was measured and enzyme levels were calculated as micromoles of *p*-nitrophenol released per gram per hour.

Sulfatase and β-glucosidase levels were measured using *p*-nitrophenylsulfate and *p*-nitrophenyl-β-D-glucoside as respective substrates and incubated for 2–24 h until color formation.

Protease levels in 1 cm^3 of sediment or from a filter of concentrated particulate material were measured using the method described in Griffiths et al. (1983), adapted from Ladd and Butler (1972). Results were expressed as micromoles of tyrosine equivalents released per gram per hour. The levels of three enzymes responsible for the hydrolysis of major plant (cellulose and xylan) and algal (laminarin) polysaccharides were measured in each sediment sample and one agglomerate sample. One cubic centimetre of sediment or a filter with the residue of 50 mL of agglomerate suspension (station 4) was mixed with 1 mL of 0.1 *M* acetate buffer (pH 5.5) and incubated with 1 mL of a 1% solution of either carboxymethylcellulose, laminarin, or xylan (Sigma Chemical Co.) and 0.2 mL of toluene at 25°C. After 17–24 h, the reaction mixture was centrifuged for 10 min and the reducing sugar content of the supernatant was determined with the dinitrosalicylic acid reagent of Bernfeld (1955). Enzyme levels were calculated as nanomoles of glucose equivalents released per gram per hour.

Results and Discussion

Over the duration of the study, marine snow agglomerates ranging in size from about 0.25 to 2500 mm^3 were seen. Agglomerates were observed to be in three forms: (1) flat, roughly circular flakes and sheets, (2) slender threadlike filaments, and (3) globular masses. Most flakes were 0.5–5.0 mm across (0.25–25.0 mm^2) by 50–100 μm thick (0.01–2.5 mm^3). Filamentous forms generally had diameters of 0.5–10.0 mm and lengths between 2 and 400 mm. Globular agglomerates were spheroidal (5.0–50.0 mm in diameter) to elliptoidal (5.0–10.0 mm in diameter by 10–30 mm long). All forms were very fragile and with only slight water turbulence were disrupted and disintegrated into many smaller fragments.

Figure 2A illustrates a typical agglomerate of Howe Sound marine snow. Figures 2B and 2C reveal that a great amount of material is accreated to form even small agglomerates. This results in a tremendously porous microstructure and a surface texture which is very roughened at the microscopic level. Hence, the actual surface area and the volume of agglomerates are very much different from what might be calculated from estimates based only on their macroscopic dimensions.

All agglomerates recovered from sample sites during the study were accretions of phytoplanktonic and zooplanktonic

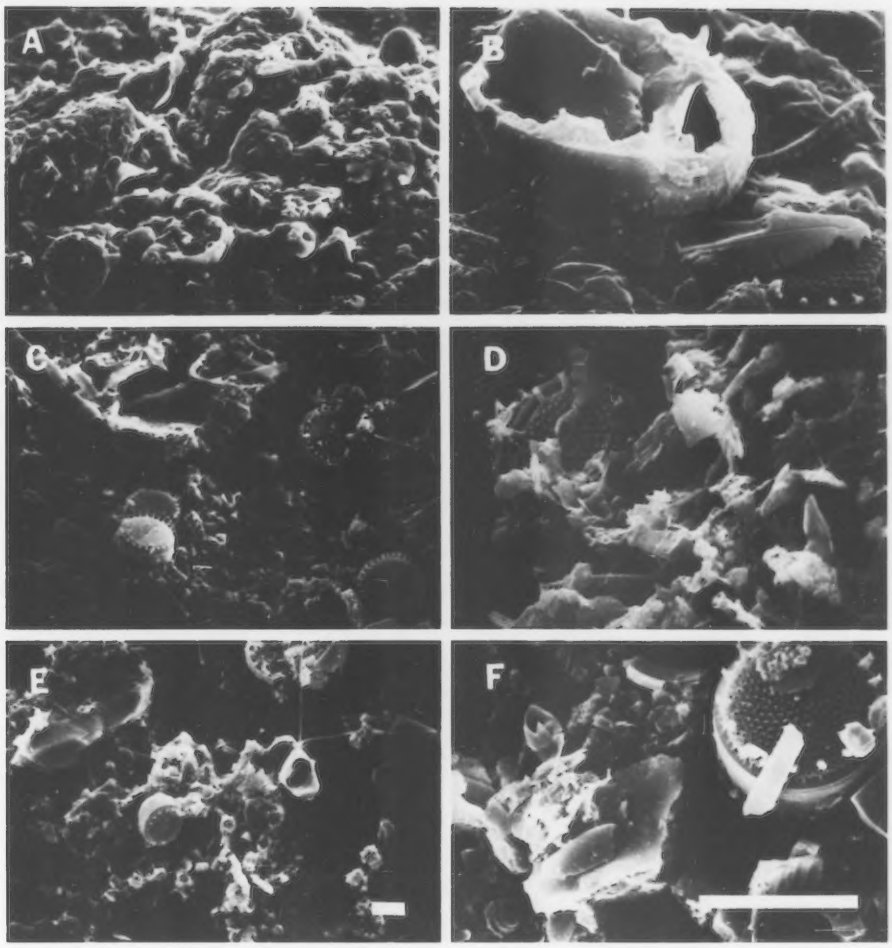

FIG. 3. Trend toward reduction in the amount of amorphous coating found adhering to agglomerates of marine snow from (A and B) station 1, 200 m, (C and D) station 2, 280 m, and (E and F) station 3, 100 m, collected in April 1984; bars = 10 μm.

cells and debris and particles of silts and clays. Many components had undergone partial dissolution and extensive physical breakage. The material adhered together by thin, amorphous interconnecting films and coatings (Fig. 2D). Ionic and electronic charge, chemical polarity and bonding, pH-related precipitation, and surface tension effects undoubtedly also affect structural cohesion (Scholkovitz 1976; Syvitski and Murray 1981; Avnimelech et al. 1982; Edzwald et al. 1974; Rashid et al. 1972), but these forces cannot be measured by microscopy.

Comparison of Fig. 2 with Fig. 3 shows that the fundamental composition of agglomerates appeared similar throughout the year and at all depths. All our samples of marine snow from various depths and times from Howe Sound, Bute Inlet, and Saanich Inlet were found to be compositionally similar when examined with SEM (data not shown). This suggests widespread general consistency in the biological and detrital components of marine snow found in water masses contiguous with the southern end of the Strait of Georgia and supports the findings of Syvitski and Murray (1981). However, other researchers have shown changes in the chemical composition of mineral components both in Howe Sound (Syvitski and MacDonald 1982) and adjacent Bute Inlet (Farrow et al. 1983; Syvitski et al. 1985).

Figure 3 demonstrates a progressive reduction in the amount of amorphous material and an increase in distinct components in marine agglomerates at three stations along a transect from station 1 to station 3. The agglomerates from station 1 (Fig. 3A and 3B) show a strikingly different structure from those from either station 2 (Fig. 3C and 3D) or station 3 (Fig. 3E and 3F). In the former the amorphous coating is more apparent and results in surface textural patterns which are larger and more rounded, giving the structure a noticeably more cohesive character. Similar observations have been reported for Howe Sound by Syvitski and Murray (1981), but the trend is not confined to the waters of southern British Columbia. Studies by Emery and Honjo (1979) and Honjo (1978), for example, reported finding more amorphous films associated with coastal

water agglomerates than with offshore agglomerates in samples taken off eastern Asia and between western Africa and the mid-Atlantic ridge.

Comparison of Fig. 2 and 3 shows that the relative amount of amorphous substance associated with agglomerates at station 2 in October 1983 (Fig. 2) appeared lower than the amount of amorphous component detected at the same station in April 1984 (Fig. 3C and 3D). In October, freshwater runoff is low, and in response to lower temperatures and shorter daylengths, the planktonic standing crop declines. In spring, freshwater runoff is higher and temperature and photoperiod increase, which results in an expanding plankton crop. We postulate that the organic matter available to precipitate with marine snow agglomerates probably shifts up or down the fjord on a seasonally cyclic basis corresponding with cycles in freshwater and terrestrially derived input, salinity, temperature, and photoperiod. The amorphous-dominated material in the upper fjord provides surfaces attractive for bacterial colonization whereas the more complex lower fjord agglomerates include numerous planktonic cells and debris. The potential significance of this has been suggested in Knight Inlet, B.C., where the distribution of benthic fauna may reflect feeding strategies related to composition of detritus (Syvitski et al. 1985).

Structural differences in the composition of marine snow cause variation in porosity, permeability, agglomerate density, surface area, and surface texture. Such variations can have significant consequences for the organism grazing on these agglomerates. While certain microbiological activities occur in marine snow in general, other specific activities may be limited to certain structural configurations within the total mass of the particulates present in the water mass.

Although SEM of agglomerates showed the absence of extensive microbial colonization, many colonies were readily cultured from the agglomerates placed on SLX agar. Rod, coccoidal, and filamentous bacteria were recovered in culture and documented by EM. These observations suggest that there is a great diversity of microorganisms associated with marine snow and that those microbes able to exploit the nutrients and microhabitats of marine snow may do so primarily as individual cells, microcolonies, or organisms intermittently associated with the agglomerates.

Results for heterotropic activity as [^{3}H]glucose uptake, bacterial counts, and carbon-based biomass and the levels of seven major hydrolytic enzymes involved in the degradation of organic matter and nutrient regeneration from agglomerate and sediment samples are listed in Table 1. The overall ranges of sediment enzymes detected in this study overlapped those reported in similar assays, except β-glucosidase, for sediments from a coastal embayment on the Aleutian Peninsula, Alaska (Griffiths et al. 1983), and the Yaquina River estuary on the Oregon coast (B. A. Caldwell, unpubl. data). In addition to differences between agglomerate and sediment samples at a given station, substantial differences were seen in many variables between stations.

Glucose uptake levels, by weight, were 2–60 times greater (station means) in the agglomerates than in the underlying sediments. The highest agglomerate to sediment ratio was found at station 2 and the lowest at station 3. Glucose uptake in the agglomerates increased seaward whereas a more complex pattern was found in the sediment. Here, uptake declined from station 1 to 2, station 3 had the highest, and the amount declined again at the most seaward station 4.

Comparison of bacterial counts and biomass, as bacterial carbon, was less clear. Greater bacterial counts were found in sediment at station 1, while numbers overlapped at stations 2 and 3. Sediment bacterial counts were not made at station 4. When expressed as bacterial carbon, the sediment biomass was higher than agglomerate biomass at station 1, much less than agglomerate biomass at station 2, and overlapped at station 3. Bacterial counts for water samples not containing agglomerates were between 2.3 and 6.8 $\times$ $10^5 \cdot mL^{-1}$, which is similar to previous reports for these waters (Albright 1983a, 1983b).

To evaluate the relative microbial activities of agglomerate and sediment samples and of the same material at different locations, glucose uptake was standardized on a per unit bacterial carbon basis (as picomoles of glucose per gram of bacterial carbon per hour). These values are listed in Table 1. Using this analysis, the range of values for the agglomerates from stations 1 and 2 (0.279–0.495 and 0.345–0.624) was much greater than the corresponding sediment ranges (0.016–0.018 and 0.050–0.083). This indicates that the microbial populations at these stations were more active in the agglomerates than those in the sediments. Station 3 produced overlapping ranges for agglomerate (0.217–0.747) and sediment (0.140–0.311) samples. One possible explanation for this is that more of the organic material at these seaward stations comes from readily usable planktonic sources, while recalcitrant terrestrial materials from river input influence the two more landward stations.

Phosphatase, which is involved in the mineralization of inorganic phosphate from organic matter, had higher levels in the agglomerate samples than in the sediments, and the highest levels were in stations 1 and 2 north of the inner sill. This may be because the enzyme is absorbed to riverine clay particles and humic materials in the agglomerates — a phenomenon known to occur in soils (Burns 1980). This is further supported by the previously mentioned decrease in amorphous material with which these enzymes may be associated, in the seaward transect from station 1 to 3.

Detectable levels of sulfatase, an enzyme responsible for the remineralization of sulfate from organic sulfate esters (Fitzgerald 1976), were found only in the sediment samples. Since this enzyme is typically inducible, its apparent absence in the agglomerate could be due to the abundance of sulfate in the surrounding seawater. In contrast, activities in the sediments could be indicative of sulfate regeneration to support anaerobic metabolism at some depth within the sediment (King and Klug 1980). Sediment sulfatase levels increased seaward to station 3, but to a greater extent than phosphatase, and then declined slightly.

Levels of β-glucosidase in agglomerate samples were detected only at stations 1 and 2 where they were 6 orders of magnitude greater than in the corresponding sediment levels. The absence of detectable enzyme levels at the two lower stations may be due to the same phenomenon that caused the rapid decline in agglomerate phosphatase activity, i.e. rapid sedimention with extensive coatings of amorphous material, including adsorbed enzymes. This is partially supported by the fact that the highest level of sediment β-glucosidase was at station 1 and the levels progressively dropped seaward.

In a limited sampling of protease and cellulase (stations 3 and 4) and laminarinase and xylanase (one concentrated sample from station 4), the levels in the agglomerates were also much greater than in the sediments, except for cellulase which was not detected in the sediment at station 4. The sediment levels of these enzymes were highest at stations seaward. This

TABLE 1. Bacterial biomass, heterotrophic activity, and hydrolytic enzyme levels, Howe Sound, C

	Station 1		Station 2	
	Agglomerates	Sediments	Agglomerates	Sedimer
Bacterial counts·g^{-1}	(6.54−10.80) × 10^{9}	(1.68−4.26) × 10^{10}	(1.29−1.47) × 10^{9}	(1.66−3.89
Bacterial biomass (μg C·g^{-1})	61.9−102	160−291	124−255	15.8−24.2
Glucose uptake (pmol·g^{-1}·h^{-1})	30.9−50.8	2.82−5.35	77.4−87.7	1.26−1.31
Glucose uptake (pmol·g bacterial biomass^{-1}·h^{-1})	0.279−0.495	0.016−0.018	0.345−0.624	0.050−0.08
Phosphatase (μmol·g^{-1}·h^{-1})	41.2−100.0	0.750−0.825	61.0−76.2	1.00−1.22
Sulfatase (μmol·g^{-1}·h^{-1})	0	0.062−0.071	0	0.146−0.15
β-Glucosidase (μmol·g^{-1}·h^{-1})	228−300	0.406−0.466	223−476	0.068−0.07
Protease (μmol·g^{-1}·h^{-1})	ND	0.031−0.035	ND	0.041−0.04
Cellulase (nmol·g^{-1}·h^{-1})	ND	25.14−67.63	ND	40.78−48.2
Laminarinase (nmol·g^{-1}·h^{-1})	ND	72.55−101.50	ND	77.0−101.
Xylanase (nmol·g^{-1}·h^{-1})	ND	116−176	ND	141−193

Sound, October 1984. ND = not determined; 0 = below level of detection.

	Station 3		Station 4	
Sediments	Agglomerates	Sediments	Agglomerates	Sediments
$56-3.89) \times 10^9$	$(1.35-3.52) \times 10^{10}$	$(1.30-1.78) \times 10^9$	$(6.86-12.70) \times 10^9$	ND
.8–24.2	118–333	124–147	65.7–110	ND
26–1.31	48.7–95.9	20.6–43.5	104–168	9.60–15.9
50–0.083	0.217–0.747	0.140–0.311	ND	ND
00–1.22	6.8–10.4	2.91–3.99	3.55–4.36	1.48–1.97
46–0.151	0	1.85–2.38	0	1.43–1.82
68–0.076	0	0.114–0.208	0	0.007–0.016
41–0.045	11.06–15.69	0.191–0.219	1.57–2.18	0.089–0.194
78–48.20	ND	87.20–114.90	6069	0
.0–101.00	ND	325–400	12640	211–470
41–193	ND	177–200	16900	208–265

may indicate a drop in the terrestrially derived material (high in cellulose) and a rise in phytoplanktonic material (high in laminarin). However, the increasing xylanase levels seaward contradict this because xylans are generally found in higher terrestrial plants.

The high glucose uptake and enzyme levels of most sediments of station 3, and to a lesser extent station 4, indicate that this is a region of major organic matter deposition supporting the benthic community. The abundant populations of echinoderms, tube works, shrimp, and fish at these two stations supported this whereas virtually no macroorganisms were seen on the sediment surface at stations 1 and 2.

The regionally widespread agglomerate character of marine snow is well documented for the Strait of Georgia and its contiguous waters. Our study finds that there is a spatial and functional diversity of microscale habitats affecting the microbiota and the metabolic processes associated with them. The significance of this variability and diversity may be easily overlooked by studies that emphasize the macroscopic features of the fjord ecosystem.

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Abundance of Larval Tanner Crabs (*Chionoecetes* spp.) in Relation to Adult Females and Regional Oceanography of the Southeastern Bering Sea[1]

Lewis S. Incze[2] and David A. Armstrong

College of Ocean and Fishery Sciences WH-10, University of Washington, Seattle, WA 98195, USA

and Sharon L. Smith

Oceanographic Sciences Division, Brookhaven National Laboratory, Upton, NY 11973, USA

Incze, L. S., D. A. Armstrong, and S. L. Smith. 1987 Abundance of larval Tanner crabs (*Chionoecetes* spp.) in relation to adult females and regional oceanography of the southeastern Bering Sea. Can. J. Fish. Aquat. Sci. 44: 1143–1156.

Abundances of larval *Chionoecetes bairdi* and *C. opilio* in the southeastern Bering Sea are compared for springs of 1978 through 1981. Geographical subareas of the shelf were designated for statistical analysis of results to accommodate variations in hydrographic conditions and circulation, distribution of benthic populations, and distribution of planktonic sampling effort. Maximum observed abundances of first-stage zoeae were 1230 and 12 697·m^{-2} for *C. bairdi* (during May) and *C. opilio* (during April), respectively. Numbers exceeding 10% of these values were common over some portions of the shelf during all years of study. Significant ($P < 0.001$) interannual differences in larval abundance were detected: in three subareas for *C. opilio* and in one for *C. bairdi*. The sharp decline in abundance of larval *C. opilio* after 1979 in two of the subareas could be accounted for by regional declines in numbers of adult female crabs. This was not true of the remaining subarea where two weak larval year-classes of *C. opilio* (1980 and 1981) and one of *C. bairdi* (1980) occurred despite an abundance of adult females. Vertical distribution of larval crabs is shown and the potential roles of advection, planktonic conditions, and reproductive output are evaluated with respect to observed patterns of larval abundance.

Les auteurs ont comparé l'abondance larvaire de printemps de *Chionoecetes bairdi* et *C. opilio* dans la partie sud-est de la mer de Béring de 1978 à 1981. Des sous-zones géographiques du plateau ont été délimitées aux fins de l'analyse statistique des résultats et de façon à tenir compte des variations des conditions hydrographiques, de la circulation, de la répartition des populations benthiques et de la distribution de l'effort d'échantillonnage du plancton. Les abondances maximales observées de zoés de premier stade étaient de 1 230 et de 12 697 individus par mètre carré pour *C. bairdi* (en mai) et *C. opilio* (en avril), respectivement. Des valeurs supérieures de 10 % à ces dernières ont fréquemment été notées au cours de toutes les années de l'étude dans certaines parties du plateau. Des différences interannuelles significatives ($P < 0{,}001$) de l'abondance larvaire ont été notées dans trois sous-zones pour *C. opilio* et dans une sous-zone pour *C. bairdi*. Le déclin abrupt de l'abondance des larves de *C. opilio* noté après 1979 dans deux des sous-zones pouvait s'expliquer par la baisse, dans cette région, du nombre de crabes femelles adultes. Cela n'était cependant pas vrai pour le reste de la zone où l'on a noté deux faibles classes annuelles de larves de *C. opilio* (1980 et 1981) et une de *C. bairdi* (1980) qui sont survenues en dépit d'une abondance de femelles adultes. Les auteurs décrivent la distribution verticale des larves de crabes et traitent des rôles possibles de l'advection, des conditions planctoniques et de la capacité reproductive dans le contexte des régimes observés de l'abondance larvaire.

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Two species of Tanner crab, *Chionoecetes bairdi* and *C. opilio* (Brachyura, Majidae), are extremely abundant from depths of 50 to 200 m on the continental shelf of the southeastern Bering Sea (Fig. 1) (Pereyra et al. 1976; Otto 1981). The species are allopatric except for this region of overlap. Here, *C. bairdi* reaches its northern limit of distribution and shows an affinity for warmer bottom water which exists along the outer continental shelf (at depths >100 m) and north of the Alaska Peninsula. *Chionoecetes opilio* reaches its southernmost latitude for the eastern North Pacific and is most abundant over the middle shelf, at depths from 50 to 100 m, where bottom water is coldest (Ingraham 1981; Kinder and Schumacher 1981). While patterns of greatest population density differ for the two species, both can be found together over a significant portion of the shelf southeast of the Pribilof Islands (Otto 1981). There is no indication that either species undertakes large seasonal migrations in this

[1]Contribution No. 712 of the School of Fisheries, College of Ocean and Fishery Sciences, University of Washington.

[2]Mailing address: National Marine Fisheries Service, Bldg. 4, 7600 Sand Pt. Way NE, Seattle, WA 98115, USA.

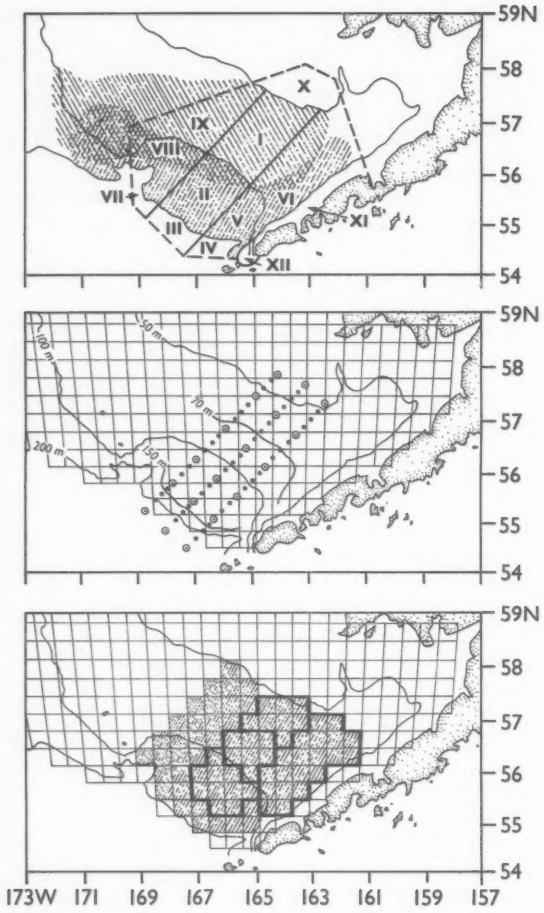


FIG. 1. Study area in the southeastern Bering Sea. Top panel shows major habitat of *C. bairdi* (positively sloping diagonal) and *C. opilio* (negatively sloping diagonal) and the geographical subareas used in statistical analyses (Roman numerals). Middle panel shows sampling grid used for assessment of benthic crabs and the principal cross-shelf planktonic sampling transects used during PROBES. The outer two transects define the boundaries of subareas I, II, and III shown in the top panel. Circled stations were sampled with MOCNESS. Principal isobaths referenced in this study are shown. Bottom panel shows grid areas (shaded) used in assessing benthic populations: middle and outer shelf domains are separated by the 100-m isobath (see middle panel); subareas (heavy outlines) are I, II, and VI (see top panel) which were further subdivided in some analyses.

region (unpubl. data; also see Donaldson 1983).

Both *C. bairdi* and *C. opilio* are predators in the benthic food web (Feder and Jewett 1981) and are the basis for one of North America's leading fisheries for crab (Otto 1981; Incze and Schumacher 1986). Their populations are surveyed annually to provide data on size, sex, and maturity of individual animals as well as estimates of their abundance (same survey as described by Otto (1986) for red king crab, *Paralithodes camtschatica*). Extensive data for Tanner crabs in this region extend from 1975 to the present and show a decline in the populations through the time period covered by this study (Otto 1981 and Fig. 2). Somerton (1981a) examined size-frequency data for female crabs (which are not commercially exploited) for 1969–79 from a subsection of the shelf south of 57°30′N latitude. He found marked fluctuations in abundance and size distribution of *C. opilio* which he interpreted as sporadic occurrences of strong year-classes which can dominate the population for several years. Variations in abundance of *C. bairdi* appeared considerably smaller.

Variations in transport and survival of larvae may be an important source of recruitment variability in adult populations. Larval survival depends on feeding conditions and predation, and both have been subjects of considerable research, primarily on larval fish (e.g. Hunter 1981; Frank and Leggett 1982; Townsend 1983; Dagg et al. 1984; Fortier and Leggett 1984; Moller 1984). Few comparable studies deal with larval decapod crustaceans, though an extensive literature exists on their behavior, development, and physiology. For example, several studies have examined energy budgets (e.g. Anger and Dawirs 1982; Dawirs 1983; Anger and Jacobi 1985) respiration and changes in metabolic substrates (e.g. Capuzzo and Lancaster 1979), and effects of temperature on growth, feeding, and respiration (e.g. Omori 1979; Anger 1983; Paul and Nunes 1983). Preliminary estimates of in situ growth and growth rates have been made for Tanner crab zoeae (Incze et al. 1984). These studies are valuable precursors for assessing feeding requirements in nature.

Only a few studies have looked at feeding by decapod crustacean larvae on naturally occurring prey, however (Paul et al. 1979; Paul and Paul 1980; Stickney and Perkins 1981; Incze and Paul 1983), and few quantitative data exist relating larval feeding to natural assemblages of organisms at natural levels of abundance. A similar dearth of information pertains to studies of predation on these larvae. Physiological indices of nutritional ("starvation") stress have been examined in crustacean meroplankton (Storch and Anger 1983; Anger 1984; Anger et al. 1985) and an amphipod (Storch and Burkhardt 1984) in the laboratory. We are unaware of published studies examining field populations of larval decapods for similar signs of stress, though this extension from the laboratory to the field has been made with larval fish (O'Connell 1980; Theilacker 1986).

Scarratt (1973), Lough (1976), Caddy (1979), Harding et al. (1982), Nichols et al. (1982), and Rothlisberg and Miller (1983) studied larval decapods in the field with respect to survival and/or interannual variations in abundance. Large variations in abundance sometimes were observed, but could not be attributed to specific causes. A number of authors have considered larval transport in the variable recruitment of crustacean populations (e.g. reviews by Ennis 1986; Phillips and McWilliam 1986; papers by Shanks 1985; Johnson et al. 1984; Botsford 1986; Sulkin and Epifanio 1986). Most studies have not been able to distinguish between effects of spawning population size or density, transport, and other causes of larval abundance patterns.

This study examines interannual variability in abundance of the planktonic larvae of *C. bairdi* and *C. opilio* in the southeastern Bering Sea. The study was conducted as part of an oceanographic investigation of the physics and lower trophic levels of this area (PROBES: see McRoy et al. 1986). We relate our findings on larval abundance to physical and biological conditions prevailing in the water column and to contemporaneous changes in abundance and reproductive condition of adult female crabs.

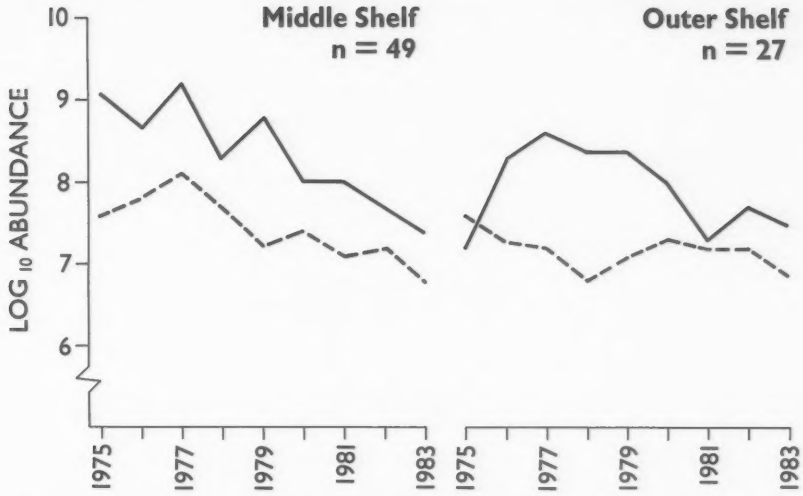


FIG. 2. Estimated abundance of adult female *C. opilio* (solid line) and *C. bairdi* (broken line) over middle and outer shelf domains of the southeastern Bering Sea (between 50 and 200 m in the study area, see Fig. 1, bottom panel). The number (n) of benthic sampling stations is given for each domain.

Materials and Methods

Larval *C. bairdi* and *C. opilio* were enumerated from 1419 plankton samples collected from the southeastern Bering Sea during April and May 1976–78 and 1980–81, June 1979–81, and July 1981. Zoeae of the two species were distinguished following criteria of Wencker et al. (1982) which add to the original descriptions by Haynes (1973, 1981). Sampling generally followed a grid during and before 1978, while cross-shelf transects prevailed during the latter three years (Fig. 1). This paper is concerned primarily with the first zoeal stage and with information collected from 1978 through 1981. Other data, which include the magalops stage, are given in Incze (1983). Sampling occurred over shelf depths ranging from approximately 45 to 1500 m.

Crab larvae were collected in all years of study with 0.6-m-diameter Bongo nets of 333- and 505-μm mesh. Nets were towed in a double-oblique fashion from the sampling depth (at least 60 m except at shallower stations) to the surface. Nets were metered individually and filtered from 150 to 250 m^3. Samples were preserved in 3–5% buffered formalin–seawater and later were examined either completely or by subsampling with a Folsom plankton splitter, depending on the abundance of larval Tanner crabs. Results were tabulated as number of larvae per 100 m^2 of sea surface.

Larval crabs also were sampled with a MOCNESS (Wiebe et al. 1976) during 1980 and 1981 (Fig. 1). The MOCNESS was equipped with 149-μm-mesh nets deployed to sample the following depth intervals, as allowed by station depth: 300–120, 120–80, 80–60, 60–40, 40–20, and 20–0 m (sometimes 20–10 and 10–0 m). Data collection followed methods of Wiebe et al. (1976), with each net filtering approximately 150–200 m^3. Samples were preserved in 5% buffered formalin–seawater and stored for later analysis. Data were tabulated for each depth interval (number of larvae per 1000 m^3) and for the water column (number per 100 m^2). Depth-stratified sampling was conducted every 6–12 h for 24 h at 12 of the MOCNESS stations. MOCNESS collections are the same as those reported for zooplankton by Smith and Vidal (1984, 1986).

Sampling efficiencies of the two types of gear were compared using data from nine sampling locations where Tanner crab larvae were present and where the bongo sampler had been deployed immediately after retrieving the MOCNESS. Comparisons were made using Tanner crab larvae, other larval decapods, and some of the more abundant copepods. Five successive double-oblique tows from 50 m to surface were made by sequentially tripping MOCNESS nets to examine variability in abundance of larval crabs using a single type of gear.

Estimates of larval Tanner crab abundance (number per 100 m^2) for each species were grouped into 12 subareas for analysis (Fig. 1). The subareas were designated to accommodate spatial differences in hydrography and circulation (Kinder and Schumacher 1981; Schumacher and Kinder 1983; Coachman 1986), distribution of adult crabs (Fig. 1), and quantity of larval data. The most complete sampling during the period 1978–81 occurred in a cross-shelf region approximately 440 km long and 112 km wide (subareas I, II, and II in Fig. 1). These three subareas are bounded by, and include data from, two cross-shelf transects used in the last two years of study. The central and primary transect of the PROBES studies is between these two transects and also is shown. All subareas were further distinguished by depth, at the 50-, 100-, and 200-m isobaths, in accordance with hydrographic changes (see below).

Data for each species, larval stage, and subarea were separated into periods of 2 wk, 1 mo, and 2 mo for various analyses. Statistical comparisons of larval abundance were made using single-factor analysis of variance (ANOVA) on log-transformed abundance data (Sokal and Rohlf 1969). The Student–Neumann–Keuls (SNK) multiple range test (Sokal and Rohlf 1969) was used to group years of similar abundance within each subregion whenever the ANOVA was significant ($P < 0.05$). A two-tailed t-test (Zar 1974) was used when only 2 yr of data existed ($\alpha = 0.05$). The final criterion of signifi-

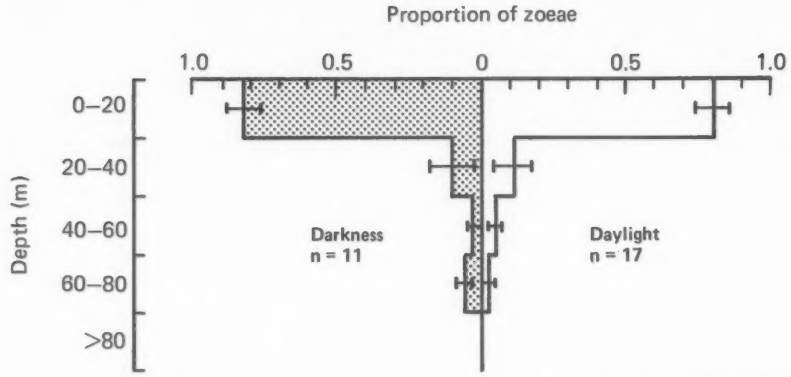


FIG. 3. Vertical distribution of zoeal stages I and II at stations sampled during darkness (21:00–03:00 meridian time) and daylight (08:00–16:00) during 1980 and 1981, middle and outer shelf domains. Data shown are mean proportions ± 1 SD; n = number of stations during each time period.

cance for multiyear comparisons was whether or not the SNK test could distinguish various levels of abundance.

Relative abundance of adult female crabs was estimated for selected areas of the shelf using the crab survey data base of the National Marine Fisheries Service, Seattle. Adult female crabs were defined by carapace widths >50 and >84 mm for *C. opilio* and *C. bairdi*, respectively. The trawl data base was used to examine the proportion of crabs of these sizes which were sexually mature (Somerton 1981b) in each year. Abundance was calculated from the estimated "area swept" by the trawl (Alverson and Pereyra 1969) assuming 100% sampling efficiency for adult crabs.

Reproductive potential of each species was estimated for specified regions based on the average size of adult female crabs in this study. The mean size of mature females was calculated for each year and for various shelf regions from the trawl data base. We used the regression equations of Haynes et al. (1976) to assign representative fecundities of 35 000 and 135 000 eggs per adult *C. opilio* and *C. bairdi*, respectively. Discussion of these values is given later. Adjustments were not made for the proportion of primiparous versus multiparous spawners (see Somerton and Meyers 1983). The average density (number per square kilometre) of adult female crabs in each subregion and the selected fecundity value for each species were used to estimate the potential number of first stage zoeae per 100 m^2 assuming complete hatching. The annual benthic surveys were conducted after most hatching had occurred, so the estimates of mature female abundance were compared with larval abundances 1 yr later. Observed and "predicted" (potential) larval abundances were compared using an index of relative abundance, A, where $A = \log[(N + 1)/(P + 1)]$ and N and P are the average number of larvae per 100 m^2 which were observed and predicted, respectively. A value of 1 was added to each independent variable to manage occasional values of zero.

Reproductive condition of female crabs was examined to identify possible biases in our estimation of potential larval production. We examined (1) the proportion of female crabs just above our size criteria which were sexually mature each year over various shelf regions and (2) clutch size (in six categories from "no eggs" to "full") for these crabs for each year and shelf region, as above.

Biotic and abiotic conditions of the pelagic environment were considered, particularly when larval abundances were far below values expected from spawning population size. Patterns of larval year-class size determined from the statistical analyses were compared with abundance of nondecapod zooplankton and with hydrographic data. Zooplankton data for 1978, 1980, and 1981 were from the same samples as the larval crabs; data for 1979 are from separate collections (Dagg et al. 1982). The original data sets were used in these analyses (Smith et al. 1982, 1983).

CTD (conductivity, temperature, depth) casts were made at virtually all sampling stations in this study. The 1-m averaged data of temperature (T), salinity (S), and density (σ_t) were used to determine depth and temperature of the upper mixed layer and temperature of the bottom water. The bottom of the upper mixed layer was defined as the depth at which σ_t changed by $0.02 \cdot m^{-1}$ and a persistent change in T and/or S occurred (see discussion by Coachman 1986). Data were not used from casts where mixed layer depth (MLD) was questionable. MLD for all stations was determined from the processed data, not from recorded profiles, and without prior knowledge of the station's location or date.

Wind direction and velocity were examined for comparison with MLD and for evaluating potential impact on transport. Estimated surface winds were derived from 6-h atmospheric pressure charts following the methods of Bakun (1973) and were compared with shipboard observations from PROBES vessels (Niebauer 1982) when they were in the area.

Results

Vertical distribution of zoeae was similar for both years of MOCNESS data (1980, 1981), for both species and zoeal stage, and for the middle and outer shelf (n = 55 stations with sufficient data for comparisons). On average, 80% of the larvae were in the upper 20 m, and more than 93% were in the upper 40 m. Data were insufficient to statistically evaluate possible differences between 20–10 and 10–0 m, though none were obvious. There were too few data to document vertical distribution during well-mixed (deep upper mixed layer) conditions. Figure 3 illustrates zoeal depth distribution during daylight and darkness selected from the 55 stations above.

Coefficients of variation (CV = mean/1 SD × 100%) for multiple samplings of Tanner crab larvae averaged 118%.

TABLE 1. Relative strength of larval year classes of *C. bairdi* and *C. opilio* based on estimated abundance. All comparisons are to be made horizontally, between years. Years of similar abundance within a subarea for each species as determined by the SNK test have common symbols: +, years of greater abundance, o, years of lesser abundance. Table of statistics shows numerator and denominator degrees of freedom (N_{df}, D_{df}), F values, and probability (P) of the F and t statistics. Blanks are left where there were insufficient data for analysis.

		Year						ANOVA table			
Species	Subarea	1976	1977	1978	1979	1980	1981	N_{df}	D_{df}	F	P
C. bairdi	I			+		o	+	2	51	12.778	<0.001
	II		+	+	+	+	+	4	74	3.899	0.0063
	VI		+	+		+	+	3	39	1.941	0.1389
	VIII	+	+	+	+			3	35	3.447	0.0269
	IX			+			+	(two-tailed t-test, P = 0.258, df = 22)			
C. opilio	I			+		o	o	2	42	14.137	<0.001
	II		+	+	+	o	o	4	73	53.574	<0.001
	VI		+	+		o	o	3	39	30.375	<0.001
	VIII	+	+	+	+			3	35	1.562	0.2160
	IX	+	+	+		+	+	4	51	2.894	0.0310

TABLE 2. Mean larval abundance (number per 100 m^2) and number of stations sampled (in parentheses) for April–June 1978–81 in three geographical subareas (I, II, and VI) identified as experiencing significant declines in larval populations of one or both species (cf. Table 1).

		C. opilio			*C. bairdi*		
		I	II	VI	I	II	VI
1978	April	172 124 (16)	35 208 (17)	24 733 (12)	101 (16)	24 (17)	1 393 (12)
	May	34 429 (6)	31 834 (13)	63 589 (4)	1 022 (6)	1 560 (13)	63 932 (4)
	June	230 (1)	10 873 (6)	5 329 (11)	0 (1)	2 122 (6)	11 951 (11)
1979	June	23 457 (3)	11 134 (11)	—	2 111 (3)	12 813 (11)	—
1980	April	0 (10)	217 (3)	—	3 (10)	6 (3)	—
	May	9 (9)	42 (5)	0 (4)	<1 (9)	2 180 (5)	2 271 (4)
	June	780 (8)[a]	67 (11)	165 (4)	794 (8)[a]	7 415 (11)	6 299 (4)
1981	April	16 (7)	128 (7)	—	0 (7)	40 (7)	—
	May	318 (13)	64 (8)	97 (8)	584 (13)	5 029 (8)	31 464 (8)
	June	212 (18)	55 (17)	267 (6)	536 (18)	5 228 (16)	22 529 (6)

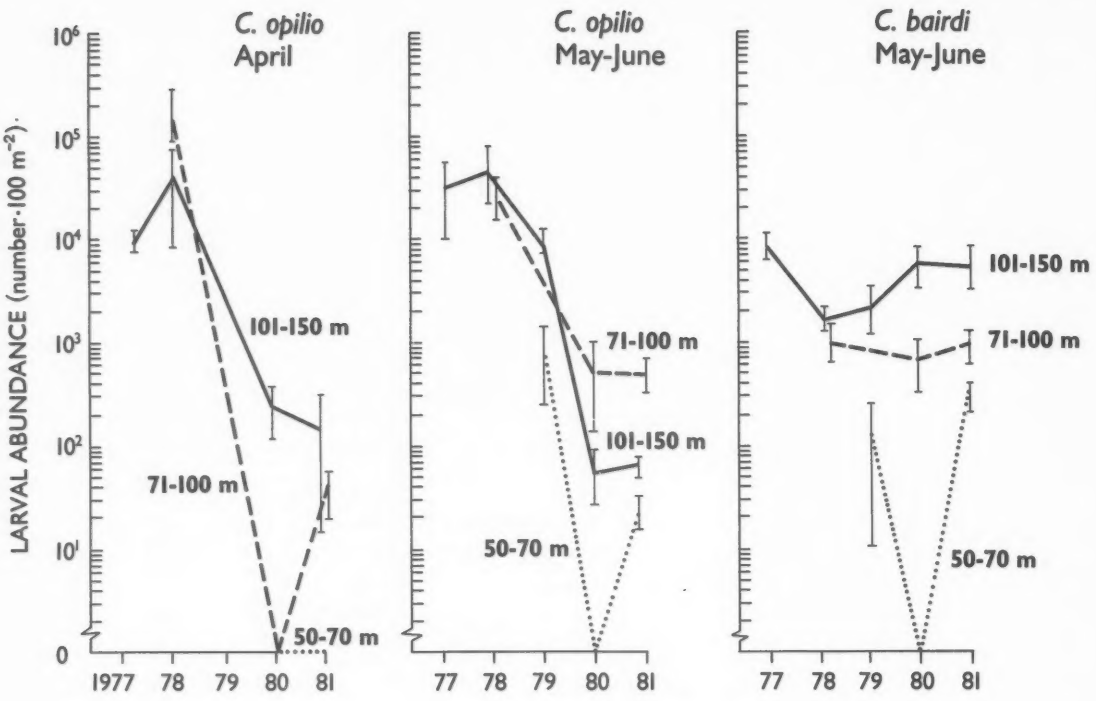
[a] Elevated abundances during June are the result of sampling from the deepest portion of subarea I (see text and Fig. 4).

These included the following comparisons: (1) samples collected from 6 to 12 h apart at the same sampling location using the same gear (n = 25 comparisons, mean CV = 82%, range = 2–141%); (2) samples taken with a MOCNESS followed by a bongo (n = 9 comparisons, mean CV = 54%, range = 4–107%); and (3) a series of sequential double-oblique tows with MOCNESS (n = 5, CV = 118%). Copepods and other larval decapods had similar variability. Coefficients of variation averaged within each subregion and month generally were less than 200%.

All larvae sampled from April through June during this study were in one of the two zoeal stages. Peak abundance of *C. bairdi* occurred during May. *Chionoecetes opilio* was most abundant during April except in subarea I during 1980 and 1981, when abundance was greatest in May. Maximum observed abundances of larval *C. bairdi* and *C. opilio* were 1230 and 12 697·m^{-2}, respectively. Densities greater than 10% of these values were common over some portions of the shelf in all years of study.

The most marked changes in larval abundance of either species occurred between April and May; changes between May and June were much smaller and data for these latter months were combined for analysis. Most June data were from the first 15 d of that month. Subregions deeper than 200 m are not considered in detail because of the unknown influence of advection along the shelf edge (Kinder et al. 1980; Paluszkiewicz and Niebauer 1984). Of the subareas shallower than 200 m, five had sufficient data for statistical analysis (Table 1). Small larval year-classes were identified by the SNK test for *C. bairdi* in subarea I during 1980 and for *C. opilio* in subareas I, II, and VI during 1980 and 1981 (ANOVA, $P < 0.001$, Table 1). Combining data for May and June did not bias analyses towards falsely classifying a year-class as small because June did not have impoverished larval populations relative to May for any of the small year-classes identified. Data for subareas I, II, and VI are shown in Table 2.

Subareas I and II were the most thoroughly sampled, and inspection of the data showed abrupt spatial changes in abundance within the subareas in 1980 and 1981. Accordingly, these subareas were further subdivided by depth as follows:



50-70 m	0	1	0	3	4	0	1	2	6	16	0	1	2	6	16
71-100 m	0	14	0	7	3	1	7	1	11	15	1	7	1	11	15
101-150 m	10	15	0	3	7	6	19	11	16	23	6	19	11	16	23

NUMBER OF SAMPLES

FIG. 4. Interannual patterns of larval abundance in cross-shelf subareas I and II according to depth: dotted line, 50–70 m; broken line, 71–100 m; solid line, 101–150 m. Data are means ± SE and are shown only where two or more samples were taken. Table at the bottom of the figure shows number of samples for each year, time period, and depth interval.

50–70 and 71–100 m in subarea I and 101–150 and 151–200 m in subarea II. These new subdivisions ranged from 92 to 112 km in cross-shelf dimension; the along-shelf (along-isobath) dimension remained unchanged at 112 km. Comparatively few larvae occurred seaward of the 150-m isobath. Inside this isobath, large interannual differences in estimated abundance were evident at all depth intervals for *C. opilio* and at the shallowest for *C. bairdi*. In general, the shallowest region experienced the most extreme changes in abundance: 3 orders of magnitude for *C. opilio* and approximately 2 orders of magnitude for *C. bairdi* (Fig. 4).

The number of adult female *C. opilio* over the middle and outer shelf domains declined from 1976 to 1983 (Fig. 2). *Chionoecetes bairdi* experienced a decline over the middle shelf during this period but was comparatively steady over the outer shelf, particularly from 1978 to 1981. Population trends for subareas I, II, and VI from 1978 to 1981 were attended in many cases by comparable changes in larval abundance (Fig. 5). In the shallowest part of subarea I (<70 m), we had only one planktonic sample from April 1978 (therefore not illustrated). This sample (12 144 zoeae·100 m^2) was 67 times the largest value sampled at any time from the same area in 1980 (n = 9) or 1981 (n = 20).

The relationship between observed and predicted larval abundance is illustrated in Fig. 6 for both species for subareas I, II, and VI. In general, including regions not illustrated here, the index of larval abundance, A, tended to be positive for *C. opilio* and negative for *C. bairdi*. The largest negative values of A, seen at shallowest depths of subarea I, indicate low larval abundances which could not be attributed to low abundance of female crabs in 1980 (both species) and 1981 (*C. opilio*).

The mean size of mature female crabs differed only slightly between years (Table 3). However, at less than 70 m, data for *C. opilio* showed a low proportion of mature compared with immature crabs >50 mm carapace width in 1979 and a low proportion of mature crabs carrying eggs in 1980 (Table 4). Such large deviations were not seen for *C. bairdi*.

The most dramatic interannual differences in zooplankton occurred in the middle shelf domain for *Pseudocalanus* spp. Figure 7 shows April and May abundances for adult females of this genus from 1978 to 1981. During late April, 1978 had an order of magnitude more adult females than 1980 or 1981. During early May, there were 5–10 times more adult females

TABLE 3. Size (carapace width, CW) for sexually mature *C. bairdi* and *C. opilio* females from various depths and years in the study area. Data include number (n) of mature female crabs measured and standard deviation (SD) of carapace widths.

		C. bairdi			*C. opilio*		
Year	Depth (m)	n	Mean CW (mm)	SD (mm)	n	Mean CW (mm)	SD (mm)
1977	50–70	239	86.2	11.5	414	64.8	5.8
	71–100	218	88.1	8.1	1009	61.5	6.7
	101–200	367	88.6	12.8	759	62.6	6.4
1978	50–70	103	89.7	9.4	142	62.3	7.8
	71–100	623	87.5	7.8	1054	61.6	6.1
	101–200	491	81.2	16.4	679	59.3	7.9
1979	50–70	167	90.8	9.0	16	64.0	3.9
	71–100	124	93.4	6.5	876	59.0	6.3
	101–200	712	83.6	10.2	558	59.5	6.3
1980	50–70	166	90.6	11.8	129	59.7	9.0
	71–100	59	87.0	11.5	106	59.9	6.8
	101–200	527	81.5	13.6	385	59.9	5.6

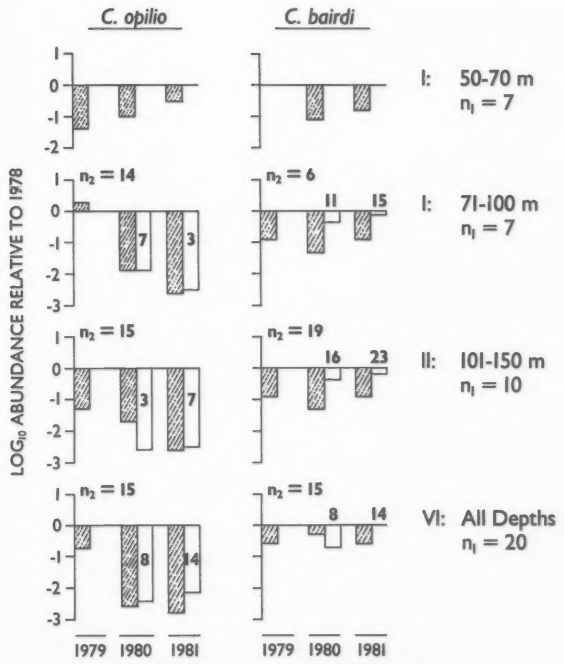


FIG. 5. Log_{10} change in measured abundance of adult female crabs (shaded bars) and larvae (open bars) at various depths in subareas I, II, and VI relative to 1978 [$\log_{10}$ (year "n" abundance/1978 abundance)]. Larval comparisons are as follows: April for *C. opilio* in I and II; May for *C. opilio* in VI; and May–June for *C. bairdi*. The number (n_1) of benthic sampling stations is given for each subarea; n_2 refers to the number of 1978 planktonic samples used in each time/subarea larval comparison; the number of planktonic samples in 1980 and 1981 used in each comparison is given over the open bars.

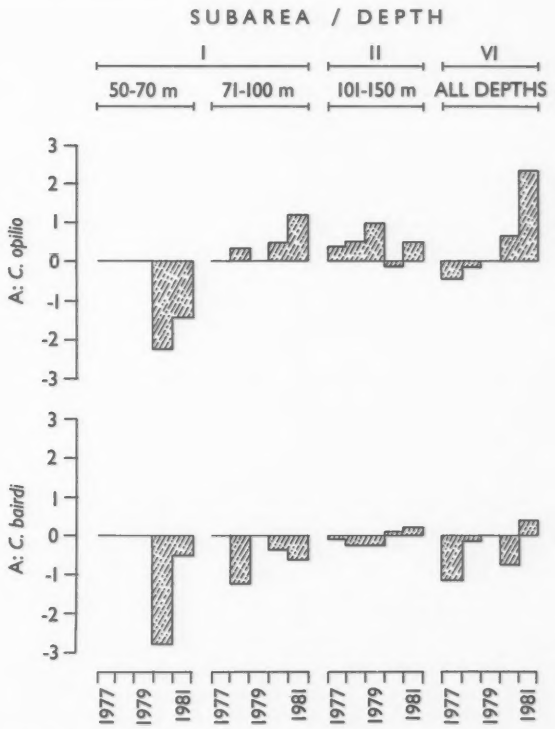


FIG. 6. Index of larval abundance (A) relative to estimated reproductive potential of the female population by year, subarea, and shelf depth: $A = \log [(N + 1)/(P + 1)]$; N and P are observed and predicted mean larval abundances, respectively.

in 1979 compared with 1980 or 1981.

Upper MLD during April of the four years showed substantial interannual differences (Fig. 8). During any particular April, MLD's were similar at outer shelf stations and were plotted as a mean for that domain. In contrast, average MLD was inversely related to depth over the middle shelf during 1979, 1980, and 1981. Areas shallower than 70 m were isopycnal to the bottom at 30% of casts taken in 1979 and at 73 and 70% of casts taken in 1980 and 1981, respectively. MLD

TABLE 4. Reproductive condition of female Tanner crabs from selected size intervals for various shelf regions and years. *Chionoecetes bairdi* are 85–89 mm carapace width (CW); *C. opilio* are 50–54 mm CW except where asterisks occur (50–59 mm CW was selected to increase sample size). Table shows number (n) of female crabs in the specified size category for which maturity data were recorded. The range of sampling dates (Julian days) is given for stations where crabs were caught; mean dates are weighted by the total number of crabs caught for each species.

Species	Shelf depth (m)	Year	n	Percent mature	Percent of mature crabs with eggs	Percent of all crabs with eggs	Range of sampling dates	Weighted mean date
C. bairdi	50–70	1977	41	96	96	92	155–187	170
		1978	23	94	87	65	142–213	159
		1979	42	100	99	99	152–233	164
		1980	23	100	74	74	134–187	148
	71–100	1977	46	98	98	95	171–188	176
		1978	163	99	96	94	142–212	152
		1979	21	100	95	95	149–233	177
		1980	17	100	94	94	133–171	146
	101–200	1977	33	98	85	84	163–211	183
		1978	48	92	89	82	140–189	168
		1979	80	92	99	92	142–214	149
		1980	82	93	93	86	132–190	154
C. opilio	50–70	1977	57	67	100	67	154–187	179
		1978	18	83	100	83	142–213	181
		1979*	16	6	100	6	152–233	190
		1980*	28	89	38	25	134–187	143
	71–100	1977	225	100	99	99	171–191	184
		1978	128	100	99	99	142–212	185
		1979	172	100	93	93	149–233	186
		1980	16	100	92	92	133–171	153
	101–200	1977	42	100	100	100	163–211	186
		1978	186	100	99	99	140–189	176
		1979	159	91	91	83	142–172	157
		1980	50	100	100	98	132–190	157

was comparatively shallow for all CTD casts made during April 1978. All middle shelf stations were stratified by May in all years of the study (Walsh 1983; Sambrotto et al. 1986).

Mean sea surface and bottom water temperatures of the middle shelf during April were coldest in 1980 and were similar during 1978, 1979, and 1981; vertical salinity gradients were greatest in 1978 and 1979 and least in 1980 and 1981 (Table 5). Good agreement existed between the Bakun (1973) wind series and shipboard observations. Six-hourly vector mean winds from geostrophic calculations at 58°N, 163°W are illustrated in Fig. 9 for 15 March to 15 June of each year. Statistics of April component winds resolved on an axis of 315° true, parallel to the 100-m isobath in the study area, are given in Table 6.

TABLE 5. Vertical salinity difference (bottom minus top), surface salinity, sea surface temperature (SST), and bottom temperature (BT) in the middle shelf domain during April. Data are from shelf areas 70–95 m deep. Surface and bottom data were averaged for the upper and bottom 4 m, respectively, before further calculations were made. Values shown are means (± 1 SD for salinity) of data from n CTD casts.

Year	n	Vertical Salinity difference ($g \cdot kg^{-1}$)	Surface Salinity ($g \cdot kg^{-1}$)	SST (°C)	BT (°C)
1978	13	0.205 ± 0.179	31.770 ± 0.108	3.11	2.31
1979	9	0.218 ± 0.151	32.098 ± 0.094	3.25	2.73
1980	8	0.072 ± 0.085	31.980 ± 0.025	1.17	1.05
1981	8	0.087 ± 0.206	31.882 ± 0.051	3.00	2.70

Discussion

Oceanographic Setting

Important hydrographic, dynamic, and biological properties of the southeastern Bering Sea vary with depth of the shelf (Coachman 1986; Codispoti et al. 1986; Smith and Vidal 1986; Whitledge et al. 1986). Regions of similar physical dynamics have been termed "domains." These are most distinct during summer and correspond to regions shallower than 50 m (coastal domain), those from 50 to 100 m deep (middle shelf domain), and those from 100 to 200 m (outer shelf). We are concerned here primarily with the middle and outer shelf domains.

The middle shelf water column is two-layered from spring (April or May) into autumn, but most or all of this domain can be isopycnal to the bottom during winter. Sea ice advected from the north (Niebauer 1980, 1983; Overland and Pease 1982) may cool the water to temperatures below 0°C, and such temperatures can be preserved in the bottom water through

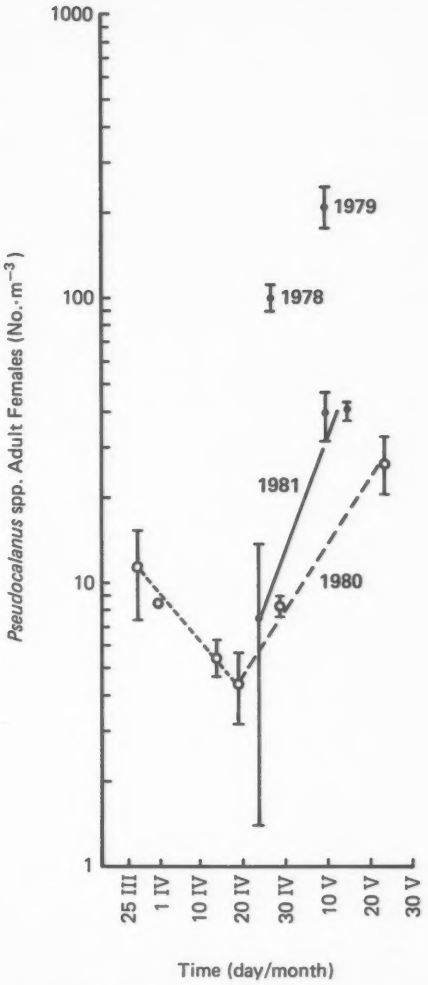


FIG. 7. Abundance of adult female *Pseudocalanus* spp. copepods in subarea I over shelf areas from 60 to 80 m deep during April and May 1978, 1979, and 1980 (open circles, broken line) and 1981 (closed circles, solid line). Data shown are means ± 1 SD. Data for 1978, 1980, and 1981 are plotted for samples collected within a maximum 3-d period (± 1 d from the date plotted); data for 1979 are from collections on 4, 9, and 17 May.

spring and summer (Coachman 1986). April is a month of transition from winter to spring and vertical density structure of the middle shelf can vary considerably between years, as seen in this study. A simple correspondence between April winds and MLD was not obvious, implying that other factors (salinity, latent heat flux, etc.) also were important. Over the outer shelf the upper mixed layer deepens during winter but remains distinct from the bottom, tidally mixed layer and showed much less variability among years.

Three low-frequency current regimes are present in the southeastern Bering Sea and coincide with the shelf sea domains. Residual flow over the outer shelf is generally to the northwest, roughly parallel to the isobaths, with speeds from 1 to 10 $cm \cdot s^{-1}$ (Schumacher and Kinder (1983) worked with records 1–8 mo long and recorded speeds mostly below 5 $cm \cdot s^{-1}$). Vector mean flows from the middle shelf are statistically insignificant (<1 $cm \cdot s^{-1}$) except near the boundaries, where mean flows are parallel to the isobaths (Schumacher and Kinder 1983). There may be significant transport parallel to the ice edge when sea ice is present (Muench and Schumacher 1985) and there is short-term (order days) transport associated with the passage of storms. The lack of nearby boundaries to the system results in current vectors rotating in response to rotating vectors of the wind field, and current speeds resulting from directionally stable winds seem to be on the order of 1% of the wind velocity (Schumacher and Kinder 1983). An along-shore flow of comparatively small volume transport follows the 50-m isobath but appears to have negligible influence on circulation of the middle or outer shelf domains (Schumacher and Kinder 1983).

Patterns of Larval and Adult Female Abundance

Tanner crab zoeae in the southeastern Bering Sea were consistently in the upper 40 m and mostly in the upper 20. They showed patchy distributions typical of other zooplankton sampled in this study and of planktonic communities in general (Wiebe et al. 1973; Fasham 1978). This variability precluded meaningful estimates of mortality from our data. However, interannual differences in abundance over subareas of the shelf were obvious and statistically significant.

The abundance of larval Tanner crabs over various regions of the shelf results from (1) reproductive output of adult females, (2) advection of larvae, and (3) factors affecting larval survival. Several conditions facilitated interpretation of our results with respect to these factors. The broad distribution of adult crabs permitted us to average data from a number of stations and concentrate on larger-scale patterns. The large size of the regions we were concerned with, in concert with frequent sampling, reduced the importance of transport as a major factor in our observations in subareas I and II. Sea ice did not extend into the middle or outer shelf in our study and the wind data offer little help in interpreting the patterns of larval abundance with respect to possible advection. For instance, April 1978 and April 1981 were the most alike with respect to winds, yet differed dramatically for larvae. The strong northerly winds (i.e. southerly vector winds) of 1980 should have favored larval transport into regions which were well sampled, so these winds do not explain the low abundance of larvae sampled in that year. Furthermore, the scales of transport indicated by the studies of Schumacher and Kinder (1983) should not have removed larvae from the large study area in the short amount of time between samplings. The long zoeal development periods, about 1 mo each (Incze 1983), enabled interannual comparisons for a single stage, thus avoiding potential problems such as different stage-specific mortality rates. Finally, surveys of benthic populations were essential; they showed that most of the precipitous decline in larval abundance after 1979 could be attributed to declines in adult female crabs. Only in the shallowest areas (50–70 m in subarea I) did we measure larval abundances far below the potential levels estimated from the abundance of adult females.

In 1980 and 1981, larval *C. opilio* were more abundant in May compared with April over the middle shelf, but not the outer shelf. This contrasts with other years, in which temporal patterns of abundance were similar for the two domains (Incze 1983). We cannot discern whether this was due to later

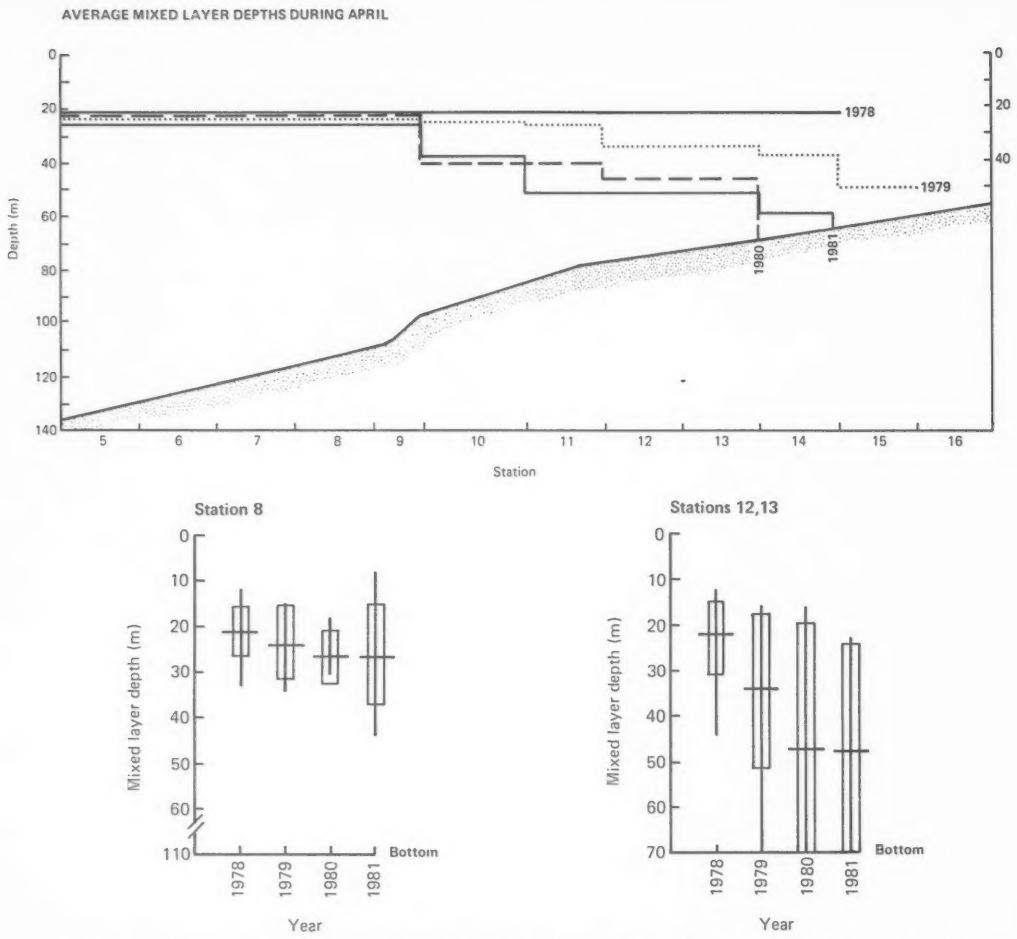


FIG. 8. Average upper mixed layer depth during April in subareas I and II, 1978–81. Top panel shows a part of the cross-shelf region with data plotted for stations shown in Fig. 1 and numbered beginning at the seaward end of the central transect. Bottom panels show the means (horizontal lines), standard deviations (vertical lines), and standard errors (boxes) of mixed layer depth for stations 8 and 12 and 13 for each year to illustrate contrasting variability of the two shelf domains.

hatching over the middle shelf in these years or to larval survivorship being skewed toward the end of the hatching period. Both explanations seem possible considering reproductive anomalies in the population and spatial and temporal changes in planktonic conditions (see later discussion). We consider advection the least likely cause of these patterns, given our data on hydrography, winds, and larval patterns.

We used an index value, A, to compare trends in abundance of adult females and early larval stages. The absolute value of A is affected by measurement problems common to all benthic and planktonic sampling, and also to two simplifying assumptions which were made. First, we assigned a single size for attainment of sexual maturity for each species when, in fact, the size at sexual maturity is quite variable (Hilsinger et al. 1975; Donaldson et al. 1980; Jewett 1981). Second, we assigned an average size and fecundity for each species which we applied to all regions and years. These assumptions seemed to work satisfactorily with healthy adult populations, producing good agreement between changes in larval and adult female abundance in many cases (cf. Fig. 5, 6).

The slightly higher index value usually seen for *C. opilio* compared with *C. bairdi* might be a function of the values we selected for fecundity, but it also is possible that this resulted from the smaller size of adult female *C. opilio*, which may make them less vulnerable to the trawl gear used in benthic sampling. In our earlier estimates of adult female populations (based on survey data), we assumed 100% sampling efficiency because no appropriate correction factor is known. Our simplifications ignore some known biological details and some unquantified factors, but they provide numbers of straightforward derivation which offer a reasonable a posteriori evaluation of one possible cause (adult population size) for the statistically significant larval differences identified by ANOVA and the SNK test.

In Fig. 5, we show changes in larval abundance relative to 1978 values without knowing in absolute terms how good a

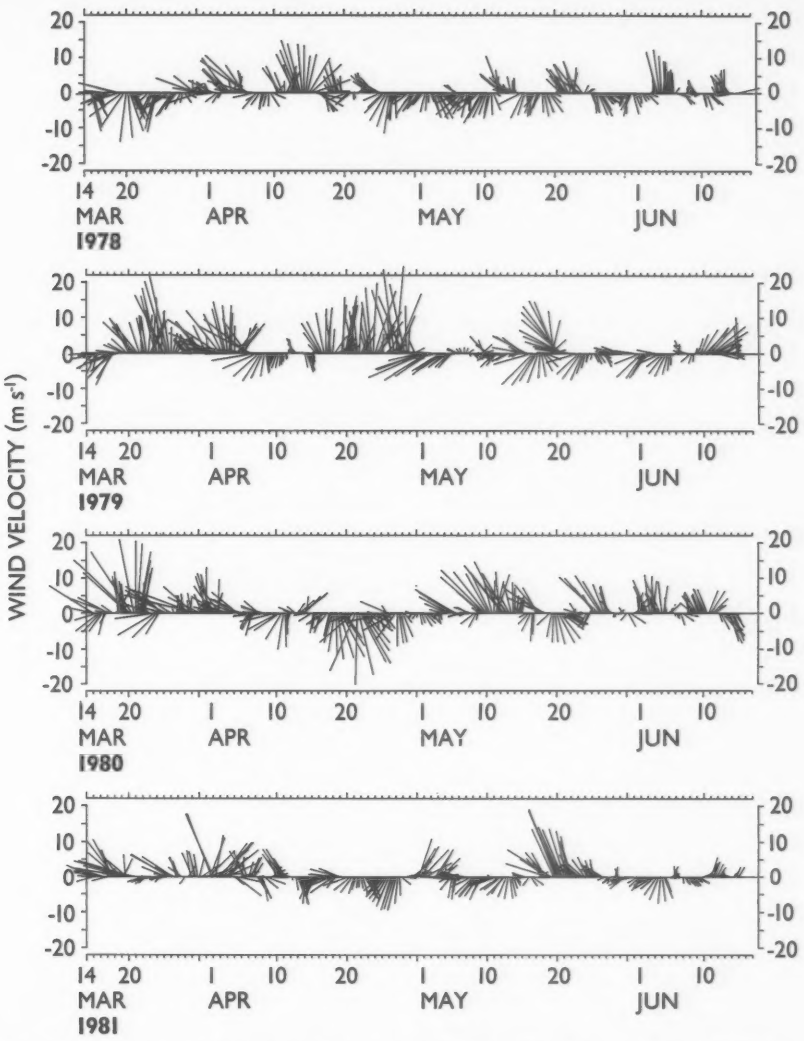


FIG. 9. Six-hourly vector mean winds (Bakun 1973) at 58°N, 163°W from 15 March to 15 June 1978–81; positive vertical axis is true north.

year 1978 was for larval survival. As with the index value, *A*, this must be viewed as a means for comparing conditions among years. Unfortunately, there were too few samples collected from the shallowest subarea in 1978 to be useful.

In subarea I, where the largest negative values of *A* were observed, it appears that zoeae (1) were rapidly transported out of the region and/or (2) were not surviving beyond the first several days of life or (3) never were produced. Any explanation must be consistent with the following: (1) no known migrations related to reproduction; (2) 7- to 12-d intervals between repeated planktonic samplings: (3) a sharp demarcation of apparent larval failure shoreward versus seaward of the 70-m (shelf depth) isobath; and (4) in one of the years (1981) only one species (*C. opilio*) was affected.

Our data suggest two possible causes. First, there is strong evidence of reproductive abnormality at shallowest depths (50–70 m) for *C. opilio*. Data on percent maturity and percent of mature crabs with eggs (Table 4) are compromised by small numbers in all of the samples during 1979 and by early timing of the survey in 1980, coldest of the 4-yr period. Since the surveys precede hatching of the larval year-class by about 10 mo, we cannot be certain that the abnormalities persisted through the normal incubation period. Nonetheless, data for these two years differ from other subareas and years and show clearly the importance of considering reproductive condition in studies which attempt to explain larval abundance patterns. These trends did not appear in the shelf-wide averages which we first examined and we cannot explain why they exhibit such marked change with depth in only one of the species. For *C. opilio*, the apparent abnormalities, if they persisted, are of sufficient magnitude to account for the low abundance of larvae in 1980 and 1981. Second, the observed patterns of

TABLE 6. Statistics of surface wind components for April at 58°N, 163°W. Principal component is for winds blowing towards 315° true; CV = coefficient of variation of wind strength along the axis of greatest variance.

Year	Component wind (° true)	Mean component speed ($m \cdot s^{-1}$)	Net velocity ($m \cdot s^{-1}$) direction	Axis of greatest variance (° true)	CV (%)
1978	315	2.33	2.36 @ 307° true	354	67
	45	−0.34			
1979	315	4.80	5.70 @ 347° true	193	69
	45	3.00			
1980	315	0.13	3.03 @ 228° true	333	68
	45	−3.03			
1981	315	−0.35	0.38 @ 157° true	206	65
	45	−0.14			

larval abundance for both species coincided with water column conditions which we discuss next.

Relationships to Measured Environmental Conditions

The apparent failure of larval year-classes at shallow depths (<70 m) in subarea I coincided with springs of low zooplankton abundance over the middle shelf and low water column stability at shelf depths less than 70 m. Over the middle shelf during spring, *Oithona* spp. and *Pseudocalanus* spp. are the most abundant copepods (Dagg et al. 1982; Smith and Vidal 1986). Adult females of the latter genus are illustrated in this paper because they are better sampled by the mesh sizes used. The most important prey of early feeding Tanner crab zoeae are not known, but probably include nauplii and perhaps early copepodid stages (some preliminary work has been done by Paul et al. (1979), Incze and Paul (1983), and Incze (1983)). We suggest that the abundance and reproductive rate of females and the size of nauplii of *Pseudocalanus* spp. make this organism an important prey for Tanner crab zoeae over the middle shelf (see calculations by Dagg et al. (1984) for larval pollock, *Theragra chalcogramma*). That copepods of this genus were in such low abundance during the last two springs in question may be related to the apparent failure of zoeae at shelf depths where the water column was isopycnal during the larval hatching period. Data on abundance of nauplii and other microzooplankton are limited for this region, but these also indicate a richer microplanktonic environment during April 1978 relative to 1980 and 1981 (Cooney et al., unpubl. report to PROBES, Institute of Marine Science, University of Alaska, Fairbanks, AK, 1978; Clarke 1984). Stability of the water column may be involved in this because of its potential effect on the vertical distribution of some species (e.g. Turner and Dagg 1983) or the rate of copepod reproduction as modified by food supply (e.g. Checkley 1980a, 1980b; Cahoon 1981; Runge 1984). It is interesting to note further that larvae of both Tanner crab species (but particularly the later-hatching *C. bairdi*) fared better in 1981 compared with 1980 over the middle shelf (Fig. 4) and that this coincided with more rapid development of the midshelf zooplankton community in 1981 (Smith and Vidal 1986).

Factors such as temperature and predators (Smith and Vidal 1986) and the effects of mixing on phytoplankton species assemblages (Sambrotto and Goering 1983) may affect the early seasonal pattern of production by *Pseudocalanus* spp. (Corkett and McLaren 1969), but these factors probably do not explain the very large contrasts observed by mid-April in this study. At these boreal latitudes, where overwintering is a necessary part of the life cycle, size of the overwintering population is of great importance and is influenced by growth and reproduction during late summer and autumn (Corkett and McLaren 1978; Colebrook 1982). The large differences in April occurred early in the spring bloom (Walsh 1983) and seemed unrelated to April temperature (Table 5). This suggests that size of the overwintering stock was an important factor determining abundance of *Pseudocalanus* spp. over the middle shelf at the time that larval Tanner crabs were hatching. We would then expect the naupliar production rate (per adult copepod) to be affected by immediate (spring time) conditions.

Conclusion

Our data show marked reductions of Tanner crab zoeae over part of the southeastern Bering Sea shelf during a 4-yr period. After adjusting for changes in adult female populations, the observed patterns of larval abundance, zooplankton abundance, and vertical mixing were consistent with an interpretation of increased mortality due to less favorable feeding conditions. This interpretation is challenged for one of the species by data indicating abnormal reproductive condition in "mature sizes" of females. We cannot resolve the relative importance of reproductive versus planktonic conditions for the two species. Nor can we knowledgeably address the question of predation, though this seems the least likely explanation for our observations. Our findings substantiate the need for information, not only on the conventional topics of larval feeding, larval predation, and planktonic biology, but also on variability of reproductive output in understanding recruitment fluctuations in crustacean populations.

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Embryonic Development and Energetic Investment in Egg Production in Relation to Size of Female Lobster (*Homarus americanus*)

Jean Attard[1] and Christiane Hudon[2,3]

Ministère des Pêches et des Océans, Direction de la Recherche sur les Pêches, Gare Maritime Champlain, 901 Cap Diamant, C.P. 15 500 Québec (Québec) G1K 7Y7

Attard, J., and C. Hudon. 1987. Embryonic development and energetic investment in egg production in relation to size of female lobster (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 44: 1157–1164.

The egg development index, dry weight, and energy content were measured on eggs from 148 ovigerous female lobsters (*Homarus americanus*) caught off îles de la Madeleine (Québec). Two modes of embryonic development were observed at stages 3 and 8 (Perkins. 1972. Fish. Bull. 70: 95–99). Stage 3 predominated among females of 71–82 mm and stage 8 among those of 83–110 mm carapace length (CL), indicating that larger females both extrude and hatch their eggs earlier than smaller females. Energy content per egg increased with female size and ranged from 4.6 cal·egg^{-1} for females 72–76 mm CL to 5.2 cal·egg^{-1} for females 107–110 mm CL. Females between 82 and 90 mm CL invested 0.155 kcal per gram of their own weight in egg production, which was the maximum value in the size range. Females smaller than 82 mm CL and larger than 90 mm CL expended proportionately less on egg production, with the difference allocated to either somatic growth in the smaller or maintenance in the larger animals. Large females produce proportionately more eggs, containing more calories per egg which hatch at an earlier time than small females. The higher energy content of eggs from large females and their earlier hatching are probably factors that enhance larval growth and survival. If so, large females contribute relatively more to recruitment than their greater fecundity.

L'indice de développement, le poids sec et le contenu énergétique des oeufs a été mesuré pour 148 homards femelles (*Homarus americanus*) provenant des îles de la Madeleine (Québec). Deux modes de développement embryonnaire ont été observés aux stades 3 et 8 (Perkins. 1972. Fish. Bull. 70: 95–99). Les oeufs les moins développés (stade 3) prédominaient parmi les femelles de 71 à 82 mm CL tandis que les oeufs les plus développés (stade 8) étaient plus fréquents chez les femelles de 83 à 110 mm CL. Cette différence indique que les femelles de grande taille portent des oeufs pondus et éclos plus tôt que les plus petites femelles. L'énergie contenue dans un oeuf augmentait avec la taille des femelles, allant de 4.6 cal·oeuf^{-1} pour les femelles de 72 à 76 mm CL a 5.2 cal·oeuf^{-1} pour les femelles de 107 à 111 mm CL. Les femelles entre 82 et 90 mm CL investissaient 0.155 kcal par gramme de leur propre poids dans la production d'oeufs, ce qui représentait la valeur maximale pour l'intervalle de taille étudié. Les femelles plus petites que 82 mm CL et plus grandes que 90 mm CL dépensaient proportionnellement moins d'énergie pour la production d'oeufs, la différence étant allouée à la croissance chez les plus petites ou au maintien chez les plus grandes. Les grosses femelles produisaient proportionnellement plus d'oeufs, pondus plus tôt en saison et contenant plus d'énergie par oeuf. L'éclosion plus précoce et le contenu énergétique plus élevé sont probablement des facteurs améliorant la survie larvaire. Si tel est le cas, la contribution relative des grosses femelles au recrutement est encore plus importante que leur fécondité accrue ne laisse présager.

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The onset of sexual maturity is an important threshold in the life cycle of the female lobster (*Homarus americanus*). From that time, energy must be shared between growth and reproduction. The timing of molt and egg extrusion varies throughout the life span of the female lobster (Aiken and Waddy 1976), presumably to achieve an optimal partitioning of energy. For most mature females, molting and spawning occur in alternate years, allowing sufficient time to store the necessary energy for either growth or reproduction (Adult-Ia and Adult-II). However, 15–20% of small females in their first year of maturity may molt and extrude eggs in the same year (Adult-Ib) (Aiken and Waddy 1980b). Very large females, on the other hand, continue to extrude eggs in alternate (or occasionally consecutive) years, while the intermolt period may last several years. These variations allow adjustments in the level of energy expenditure allocated to reproduction in conjunction with decreasing growth rate as the females become larger.

The gradual shift of resources from somatic growth to reproduction results in the well-documented increase of fecundity

[1]Present address: Laboratoire de Zoogéographie (Génétique), Université Paul-Valéry, B.P. 5043, 34032 Montpellier cedex (France).

[2]Present address: Arctic Biological Station, 555 boul. Saint-Pierre, Sainte-Anne-de-Bellevue (Québec) H9X 3L6.

[3]Author to whom correspondence should be addressed.

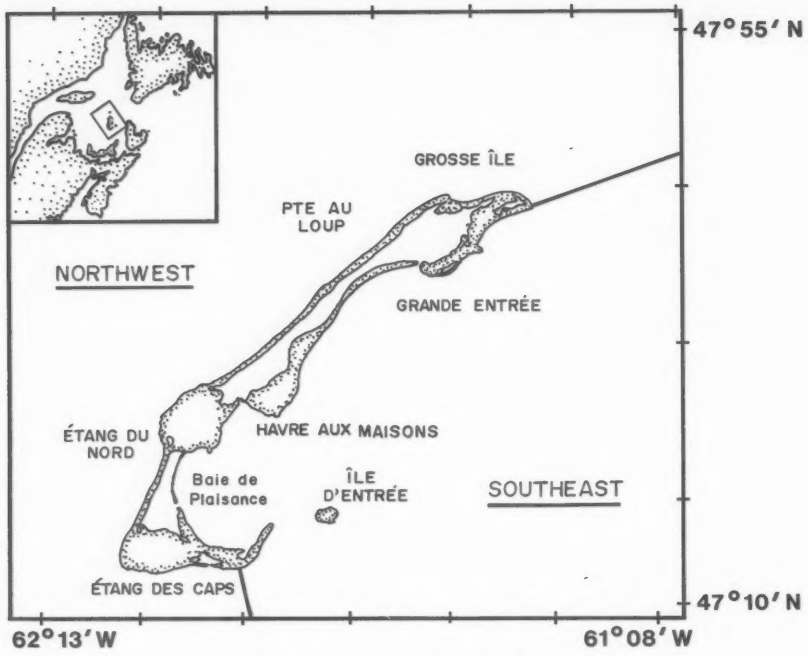

FIG. 1. Location of sampling sites off îles de la Madeleine.

TABLE 1. Summary of sampling design.

Date	Number of individuals (CL, mm) Northwest	Southeast	Analysis
13–15 May 1985	29 (79.9–109.7)	92 (72.5–94.1)	Embryonic development and energetic content
23–29 May 1985	27 (78.6–105.5)	—	Energetic content

with size of female lobster (Herrick 1909; Saila et al. 1969; Squires 1970; Perkins 1971; Ennis 1981; Campbell and Robinson 1983). The concurrent increase in weight of individual eggs with increasing fecundity (Latrouite et al. 1984) also reflects the proportionately higher total energy expenditure associated with increasing female body size.

Fecundity data may be used in the development of models of maximum sustained exploitation of lobsters of the smallest possible size commensurate with maintenance of the greatest possible egg production (Campbell and Robinson 1983; Attard 1985; Campbell 1985; Ennis 1985). Variable proportions of egg production may thus be protected from the fishery, and it is important to determine what degree of protection will assure the best continuing recruitment. For instance, the protection of all ovigerous females might not increase recruitment if the larvae produced by small females have a low biological viability due to energetic constraints.

This study is an attempt to understand the interactions between the time of egg extrusion and the number, size, and energy content of eggs produced during the life span of female lobsters. This was facilitated by the comparison of lobster populations from the two sides of îles de la Madeleine. These populations have different size structures, and fecundity (Axelsen and Dubé 1978; Dubé 1984; Attard 1985), which together give us a broad size range to observe. The relationships between the numerous factors influencing reproduction may ultimately have effects on the survival rates of larvae produced by females of different sizes.

Materials and Methods

Sampling of Ovigerous Females

Embryonic development was studied on eggs from 121 females from îles de la Madeleine. Of these, 92 were from the southeast side of the islands (île d'Entrée, Grande Entrée, Havre aux Maisons) and 29 were from the northwest side (Grosse Île, Pointe au Loup, Étang du Nord, Étang des Caps) (Fig. 1). Females from both areas were captured between 13 and 15 May 1985. Energy content of eggs was measured on these individuals and on 27 others caught on the northwest side of the islands from 23 to 29 May 1985 (Table 1). All berried females were obtained from local fishermen and immediately placed in individual plastic bags to avoid egg loss due to handling and transport.

The eggs examined in this study are assumed to have been extruded in 1984, since sampling took place a least 6 wk before the egg extrusion period in îles de la Madeleine (Attard and Fradette 1985). Carapace length (CL, millimetres) was measured from the base of the eye socket to the posterior margin of the carapace parallel to the middorsal line. Wet weight of females was calculated following a length (CL) – weight (W_f, grams) relationship for nonovigerous females (Anonymous 1978, cited in Gagnon et al. 1984) (r and n not

given):

(1) $W_f = 10^{(1.203+0.0176CL)}$

Egg Development Index

All eggs were removed from live females and preserved in 5% formalin for 2 d. After preservation, a sample of 10 eggs was taken from each brood to measure eye length and width to the nearest micrometre (Perkins 1972) and to calculate Perkins' eye index (PEI) from the mean values. The values of the egg development index were divided into ten 50-μm stages, from 1 (eye index varying from 51 to 100 μm) to 10 (eye index greater than 501 μm). The eye index reaches about 560 μm at hatching. Stage 0 corresponds to eggs without any eye pigmentation.

Fecundity

Formalin-preserved eggs were rinsed in fresh water, oven-dried at 50°C for 20 h, and rubbed over a screen of 850-μm mesh. The mean dry weight of one egg (W_e) was calculated based on the weight of two subsamples of 150 eggs. Weighing the brood allowed estimation of the total number of eggs per female. The following equations relating fecundity (F) and female size (CL) were obtained (Attard 1985):

(2) $F = 512.844CL - 30029.79$ ($r = 0.766$; $n = 50$) northwest

(3) $F = 462.099CL - 26670.20$ ($r = 0.745$; $n = 74$) southeast.

The number of eggs calculated underestimated the number actually produced because of natural losses over the 9- to 11-mo incubation period. The average egg loss equals 36% (Perkins 1971). Considering that sampling took place 1 or 2 mo before hatching (Attard and Fradette 1985), we estimated egg loss at approximately 30%. Egg production was estimated for each female by multiplying the number determined by 1.3. This correction was necessary to evaluate the total energetic investment in the brood at the time of extrusion. The variable percentage of extruded but nonattached eggs (Talbot et al. 1984) was neglected.

Energy Content

The energy content of eggs was measured using a Phillipson-type oxygen bomb calorimeter (Gentry-Wiegert Instruments, Aiken, SC) calibrated every 15–20 analyses. Each sample was analysed twice, and the mean value was used for further calculations. Results are expressed in kilocalories per gram (E_b) or calories per egg (E_e) for dry egg material, kilocalories for total energetic investment (E_t), and kilocalories per gram of female weight for relative energetic investment (RE_t).

Experimental variability was determined from nine egg samples taken at random every 12–15 analyses. Error in energetic measurement remained between 2.4 and 9.1%. Such variation might be related to differing ash content (Williams and Robins 1979) and/or to methodology (see the review of Paine 1971).

Results

Embryonic Development

In the May 1985 samples, egg development stages 0–9 were present in females from both sides of îles de la Madeleine (Fig. 2A, 2B). Stage 10, indicating eggs close to hatching, was not observed. Eggs with very little development (i.e. no eye pigment, stage 0) were found on 12–14% of the females in both areas. The eggs presented two modes of development, at stages 3 and 8, corresponding to egg development indices (PEI) of 151–200 and 401–450 μm, respectively. On the northwest side, stage 8 was proportionately more important than stage 3 whereas the inverse was observed on the southeast side. Examination of the size frequency histogram of the females carrying these eggs shows that the less-developed eggs (stage 3) were significantly more frequent in the group of small females (71–82 mm CL), while the more-developed eggs (stage 8) were mostly carried by large females (83–110 mm CL) ($\chi^2 = 9.88$; df = 1; $p < 0.01$) (Fig. 2C, 2D).

Eggs from ovigerous females sampled in 1981 on both sides of the islands also showed two modes in embryonic development (Attard and Fradette 1985). As in this study, the differences observed between the two sides of îles de la Madeleine were related to the differences of population structures (Bergeron 1967; Axelsen and Dubé 1978; Lamoureux et al. 1983). Consequently, the data from both sides of the islands were analysed as a single set covering the entire range of female lobster sizes. The dry weight and energy content per egg were first investigated to determine their relationships with female size and embryonic development. We then calculated the total energetic investment in brood production with size of female lobster.

TABLE 2. Pearson r correlation matrix between ln-transformed variables. Data from both sides of the islands are grouped. Values in italics are very highly significant ($p < 0.001$).

	CL	F	PEI	W_e	E_e	E_t
Female size (CL)	1.00					
Fecundity (F)	*0.798*	1.00				
Egg development index (PEI)	*0.314*	*0.385*	1.00			
Dry weight per egg (W_e)	*0.298*	0.123	0.118	1.00		
Energy per egg (E_e)	*0.226*	0.036	−0.023	*0.785*	1.00	
Total energetic investment in brood (E_t)	*0.814*	*0.938*	*0.359*	*0.386*	0.379	1.00

Dry Weight per Egg

The dry weight per egg (W_e) varied from 620 to 1000 μg, with an average value of 800 μg ($n = 144$; SD = 78.5). As was reported for the European lobster (*Homarus gammarus*), large females tended to produce heavier eggs than small females (Fig. 3) (Latrouite et al. 1984). If the eggs from females of both sizes developed at the same temperature, larvae produced by large females could benefit from a supplemental energy store during the early posthatch period (Sasaki et al. 1986). The egg development index and egg dry weight were not correlated in this study (Table 2), although a progressive decrease of egg dry weight as eggs developed was previously reported (Pandian 1970b; Sasaki et al. 1986).

Energetic Value

The energetic value of the brood (E_b) ranged between 5.07 and 7.18 kcal·g^{-1}, with an average value of 6.09 kcal·g^{-1}

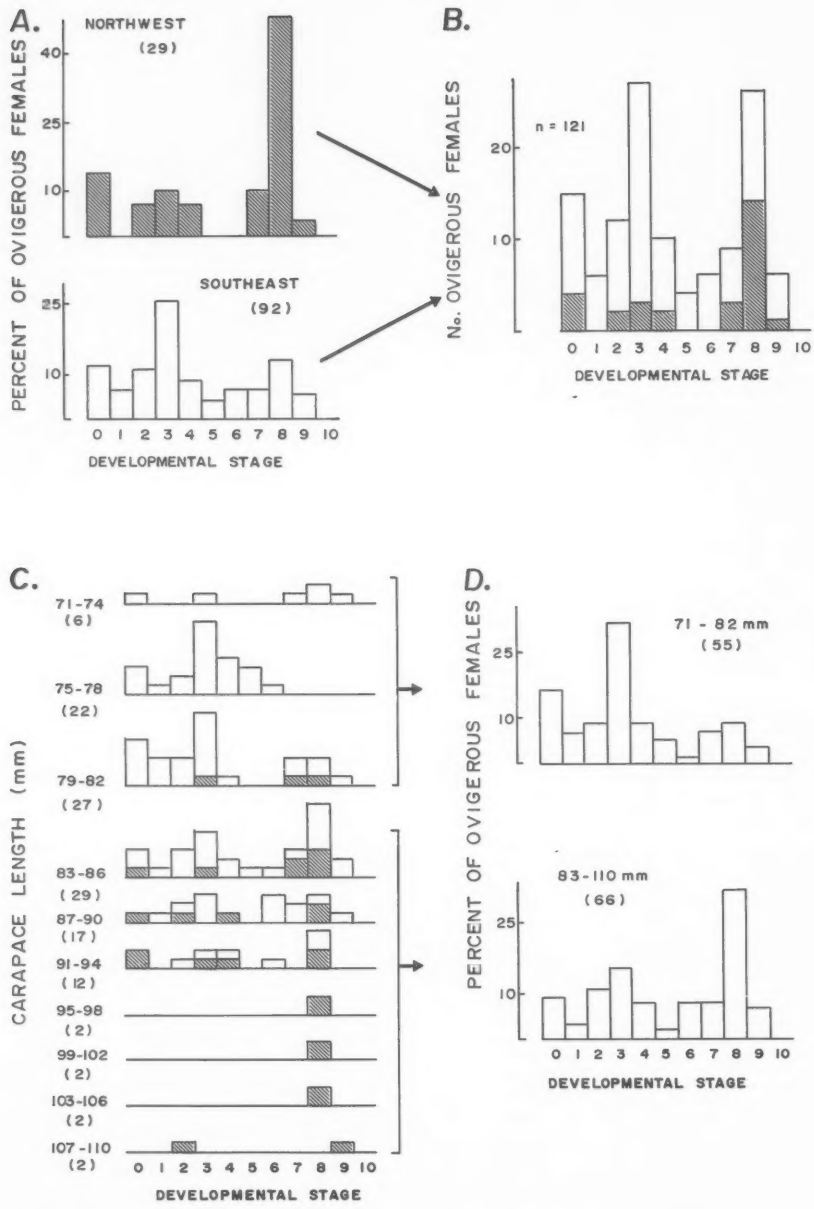

FIG. 2. Frequency histograms of embryonic development stages for the (A, B) southeast and northwest sides of îles de la Madeleine, (C) all individuals as a function of female size, and (D) two female size groups.

(Table 3). E_b was correlated with the egg development index, indicating a decrease in energy content per egg with maturation (Fig. 4). Between stage 1 and the approach of eclosion at the end of stage 10, the rate of energy consumption, as indicated by the slope of the relationship in Fig. 4, was around 6%. Energy consumption rates of 15 and 10% were obtained experimentally for *Homarus americanus* (6.6–5.6 kcal·g^{-1}) (Pandian 1970b) and *Homarus gammarus* (6.2–5.6 kcal·g^{-1}) (Pandian 1970a), respectively. The lower rate we observed could be attributed to lower energetic demands at winter temperatures than under Pandian's (1970b) experimental conditions (14°C). The energy content per egg (E_e) increased from 4.6 cal·egg^{-1} for females 72–76 mm CL to 5.2 cal·egg^{-1} for females 107–111 mm CL (Table 3).

TABLE 3. Energetic value of egg material as a function of female size.

Size, CL (mm)	Number (n)	Mean energetic value of egg material (SD)	
		E_b (kcal·g^{-1})	E_e (cal·egg^{-1})
72–76	14	6.09 (0.35)	4.6 (0.5)
77–81	44	6.05 (0.38)	4.8 (0.5)
82–86	43	6.20 (0.47)	4.9 (0.7)
87–91	23	6.06 (0.40)	4.9 (0.4)
92–96	11	6.13 (0.61)	5.0 (0.7)
97–101	7	5.99 (0.72)	5.2 (0.6)
102–106	4	5.93 (0.74)	4.9 (0.7)
107–111	2	5.53 (0.39)	5.2 (0.3)
Overall	148	6.09 (0.45)	

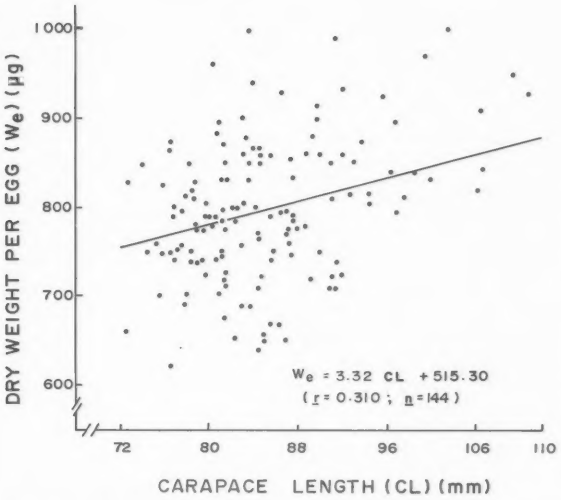

FIG. 3. Relationship between individual egg dry weight (W_e) and female lobster size (CL).

Energetic Investment in Egg Production

The total amount of energy invested in egg production (E_t) varied between 37.06 kcal for a female of 76.2 mm CL and 219.42 kcal for a female of 108.7 mm CL. The increase in total energetic investment with size reflected the higher fecundity of large females (Fig. 5A).

The relative energetic investment per unit of female somatic weight (RE_t) was highest for females 82–90 mm CL, over which sizes they invested 0.155 kcal·g^{-1} in brood production (Fig. 5B). For the smallest (72–74 mm CL) and the largest (107–110 mm CL) females, RE_t was around 0.15 and 0.14 kcal·g^{-1}, respectively.

The relationships between female size and developmental stages, dry weight of eggs, and fecundity are shown in a block diagram including Pearsons' correlation coefficients (Fig. 6). The links indicated between variables are those persisting after removal of the influence of concomitant variables using partial correlation coefficients (path analysis: Sokal and Rohlf 1981). For instance, the relationship between energy per egg and the energetic value of brood ($r = 0.379$; $p < 0.001$; Table 2)

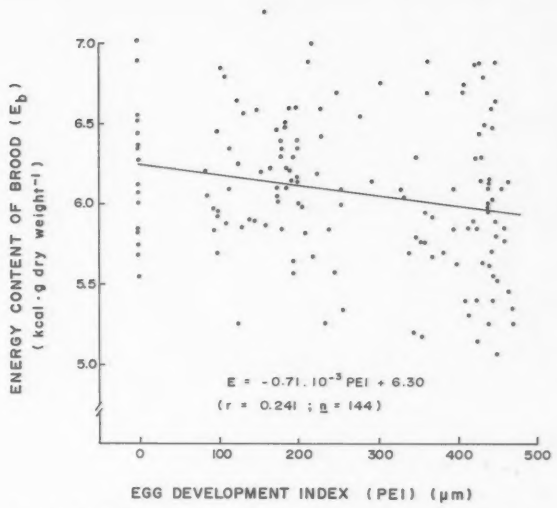

FIG. 4. Relationship between energy content per gram of brood (E_b) and egg development index (PEI).

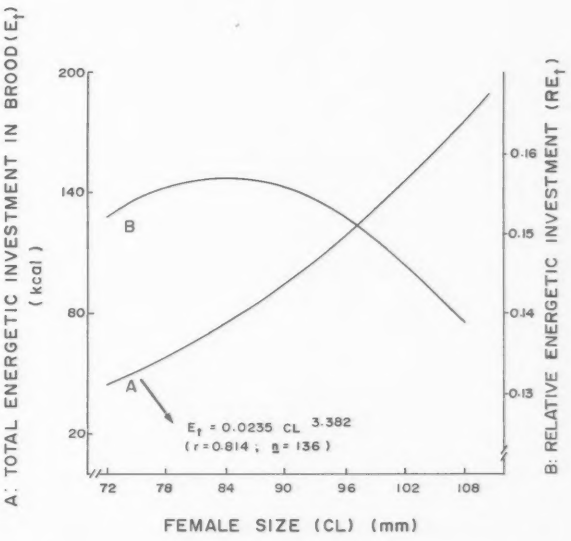

FIG. 5. Variation of (A) energetic investment and (B) relative energetic investment in egg production in relation to size of female lobster (CL).

disappeared when the effect of dry weight per egg was removed using partial correlation ($r = 0.131$; $p = 0.131$). The correlation between the energy and dry weight of each egg is very good ($r = 0.785$), in part because the former was calculated using the latter. On the diagram, therefore, only the stronger relationship of W_e with CL and E_t is shown (Fig. 6).

Two factors have a direct influence on the energetic investment in the brood (E_t) and each is related to female size: the dry weight of individual eggs and the number of eggs. Comparison of the correlation coefficients (Table 2) indicates that the increase in fecundity with age contributes greatly to the

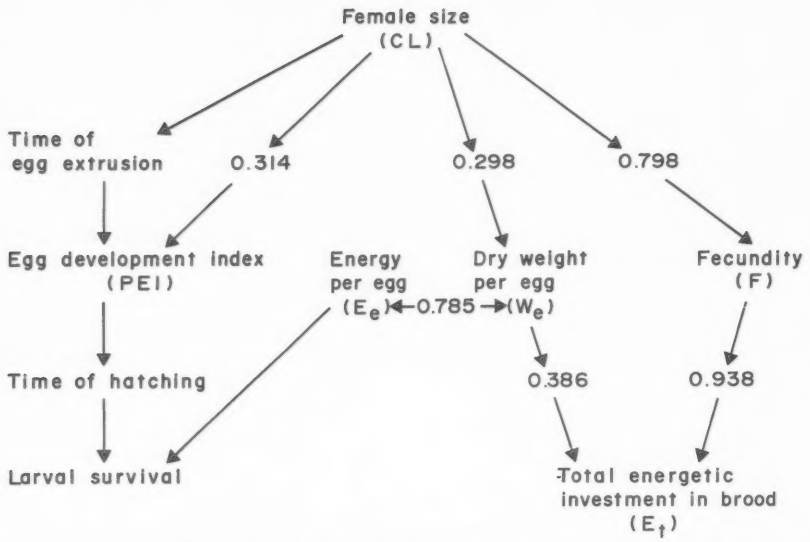

FIG. 6. Relationships between female size and egg development, energy per egg, and fecundity. All correlations are very highly significant ($p < 0.001$).

increase in energetic investment during the life of a female lobster.

A significant correlation between the egg development index and energetic investment in brood ($r = 0.359$; $p < 0.001$; Table 2) was also observed as a potential additional factor. However, the relationship disappeared when the influence of female size and number of eggs was removed using the partial correlation coefficient ($r = 0.087$; $p = 0.171$). This indicated that the initial relationship was due to the influence of a common factor, namely the strong influence of size of the female on egg development and on number of eggs.

Discussion

Embryonic Development

Female size was related to egg development, with smaller females carrying less-developed eggs. From the study of Dubé and Grondin (1985), one may assume that ovigerous females smaller than 82 mm CL are first time spawners (Adult-I) whereas most of the larger females had spawned previously (Adult-II).

The difference in egg development with female size may be explained by different times of egg extrusion. Adult-I females can be divided into two groups, one spawning the year following their molt to mature size (Adult-Ia) and the other molting and spawning in the same year (Adult-Ib) (Aiken and Waddy 1980b). For adult-Ib females, egg extrusion is delayed and occurs in late summer (Aiken and Waddy 1980a). The two egg development modes could be the consequence of two spawning periods, stages 1–4 corresponding to a late extrusion by adult-Ib females and stages 6–10 corresponding to females with the usual cycle in which molt and extrusion alternate each year (Adult-Ia and II). The frequency of early developmental stages (stages 1–4) appears to be directly related to the proportion of females having molted and extruded eggs in the same year (Adult-Ib). From Fig. 2B, we estimated that 45% of all ovigerous females can be considered as Ib adults. With all ovigerous females divided into size classes (Fig. 2C), the Ib adult group was found to comprise 56% of females between 71 and 82 mm CL and 36% of females between 83 and 110 mm CL. Ennis (1980) estimated that recently molted ovigerous females represent up to 38.5% of all ovigerous females, depending on site and year. These new-shelled ovigerous females were considered as Adult-I, since they are mostly at the lower end of the size range sampled. Robinson (1979) estimated 89.3% of 28 ovigerous females sampled to be Adult-Ib.

The occurrence of females carrying stage 0 eggs could result in part from late spawning in the previous winter. This would suggest that, although most of the spawning takes place in July–August (Attard and Fradette 1985), spawning could occur as late as October. In addition, some of the stage 0 eggs might be attributed to females (up to 15%, Ennis and Collins 1983) entirely losing their brood during the incubation period, or to unfertilized eggs.

In addition to differences in the time of egg extrusion, the range of embryonic development observed in early May could be related to the temperature regime to which the eggs were subjected during the early months of their development (Perkins 1972). In southern Bay of Fundy, ovigerous females migrate seasonally between shallow and deep water in order to gain extra heat units ensuring complete egg development (Campbell 1986). That study also showed that egg development remained more or less constant throughout winter and early spring and that the final stage of development was quickly reached in early summer. Ovigerous females from îles de la Madeleine could possibly achieve such effect by migrating inside the lagoons or in shallow coastal waters in June–July.

Time of Hatching

The wide range of egg development stages near the end of

the incubation period could explain the 2-mo duration of the hatching period off îles de la Madeleine (Templeman 1940). The expected date of egg hatching was calculated using Perkins' (1972) equation relating local ambient temperatures (Hudon et al. 1987) to the average egg development rate. Hatching of the well-developed eggs (stages 6–9) should take place around July 20 (Attard and Fradette 1985), which corresponds to the early occurrence of stage I larvae off îles de la Madeleine (Hudon and Fradette 1985). The least-developed eggs (stages 1–4) should hatch around 15 September, about 7 wk later. This latter estimate does not take into account the relatively faster development rate observed for new eggs (Perkins 1972), or the possible migration of small ovigerous females to shallow and warm waters. Such effects would allow less-developed eggs partially to catch up with well-developed eggs, thus reducing the delay between the two peaks of hatching. Indeed, the seasonal occurrence of stage I larvae in Baie de Plaisance showed two peaks of hatching in mid-July and mid-August (Hudon et al. 1986) and could result from the two modes in egg development.

Relative Energy per Unit of Female Weight

The dome-shaped curve of relative energetic investment with female size (Fig. 5B) can be partially explained mathematically, by the division of two logarithmic functions bearing different parameters subjecting the extreme values of the range to a distortion. The biological explanation is that a female increases in weight faster than in fecundity, resulting in a proportionally higher fecundity for the females in the 82–90 mm CL size range than in larger animals. Females smaller than 82 mm CL, also corresponding to Adult-I, would have a lesser investment in reproduction per unit of weight due to the high proportion of their total energy budget still allocated to somatic growth. Towards the other end of the size range, females larger than 90 mm CL exhibit a proportionately higher weight gain at each molt, which increases the basic maintenance energy level and decreases the relative investment in reproduction. This result suggests "some unidentified metabolic constraints" (Peters 1983) on requirements for basic maintenance of large animals. However, the contribution of females >90 mm CL to egg production in the population remains important, with 10% (southeast) to 40% (northwest) of îles de la Madeleine total egg production (Attard 1985).

In summary, three factors support the important contribution of large females to recruitment. Firstly, large females can extrude and fertilize eggs even in the absence of molt due to multiple fertilizations (Aiken and Waddy 1980b). Secondly, eggs extruded by most females larger than 83 mm CL hatch in early summer, and therefore benefit from high water temperatures (Hudon et al. 1986), and thus better survival (Caddy 1979). Thirdly, larvae hatching from the heavier eggs produced by large females could benefit from more energy during the posthatch period (Sasaki et al. 1986). This could allow the larvae to prolong the period of starvation prior to the beginning of active feeding and would be of considerable adaptive advantage for their early survival.

Acknowledgments

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Aluminum, Iron, Zinc, and Lead in Bog Waters of Northeastern North America

Noel R. Urban and Steven J. Eisenreich

Department of Civil and Mineral Engineering, University of Minnesota, Minneapolis, MN 55455, USA

and Eville Gorham

Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN 55455, USA

Urban, N. R., S. J. Eisenreich, and E. Gorham. 1987. Aluminum, iron, zinc, and lead in bog waters of northeastern North America. Can. J. Fish. Aquat. Sci. 44: 1165–1172.

Aluminum, iron, zinc, and lead were measured in 37 surface waters from 24 bogs along an east to west transect from Manitoba and Minnesota to Maine and the Atlantic Provinces of Canada. The major influence upon Al and Fe is atmospheric deposition of soil particles. Zinc and Pb are derived primarily from air pollution. Concentrations of Al and Fe in bog waters greatly exceed those in regional atmospheric precipitation; dry deposition and evaporative concentration are the likely explanations. Concentrations of Zn and Pb are less than those in precipitation, presumably due to sorption by peat.

Les teneurs en aluminium, fer, zinc et plomb dans 37 plans d'eau de 24 tourbières ont été déterminées le long d'un transect est–ouest allant du Manitoba et du Minnesota au Maine et aux Provinces atlantiques canadiennes. Le dépôt de particules de sol provenant de l'atmosphère est le principal paramètre influant sur les teneurs en Al et Fe. Le Zn et le Pb ont surtout pour origine la pollution atmosphérique. Les concentrations de Al et de Fe dans les eaux des tourbières excédaient de beaucoup celles des précipitations régionales; ce phénomène s'explique probablement par le dépôt par voie sèche et la concentration par évaporation. Les concentrations de Zn et de Pb sont inférieures à celles des précipitations, probablement à cause d'une sorption par la tourbe.

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Bogs are peatlands whose surface hydrologic and mineral inputs come primarily from the atmosphere (Gorham 1953). As a result, close correspondence often is observed between the magnitude of rates of atmospheric deposition of elements and the magnitude of element pools and cycling rates within bogs. Thus, the major-ion composition of bog water reflects regional precipitation chemistry (Gorham 1961; Gorham et al. 1985), and rates of nutrient cycling in bogs are closely coupled to rates of atmospheric deposition (Urban et al. 1987). Moss and peat from bogs have been used to indicate current and historical atmospheric deposition of trace metals (Ruhling and Tyler 1971; Pakarinen and Tolonen 1976, 1977; Furr et al. 1979; Glooschenko and Capobianco 1978; Glooschenko et al. 1981), nitrogen (Mattson and Koutler-Andersson 1955; Ferguson et al. 1984), and sulfur (Pakarinen 1981; Percy 1983; Ferguson et al. 1984). However, we know of no studies indicating how trace metal concentrations in bog waters are influenced by atmospheric deposition.

Several processes within peatlands might be expected to remove trace metals from bog water and obscure relations between atmospheric deposition and surface-water concentration. First, metal sulfides may precipitate in anaerobic peat where sulfide concentrations are elevated. Pyrite and acid-volatile sulfides have been found in peat (Tarleton et al. 1984; Behr 1985; Wieder et al. 1985; Urban 1987), and solubility calculations suggest that CuS may precipitate in a Minnesota bog (Schurr 1983). Second, *Sphagnum* moss and peat have high capacities for adsorbing and complexing trace metals (Malmer and Sjors 1955; Puustjarvi 1959, 1968; Bunzl et al. 1976; Bunzl and Schulz 1980) and have been shown to reduce trace metal concentrations in waters leaching from mining wastes and soils (Coker and DiLabio 1979; Eger et al. 1981). Trace metal partition coefficients between peat and bog water range from 300 to 14 000 (Pakarinen and Tolonen 1976; Aaby et al. 1979; Hemond 1980; Schurr 1983). Because of the high affinity of trace metals for the solid phase, it has been assumed that trace metal profiles in peat are accurate records of historic patterns and magnitudes of atmospheric deposition (e.g. Livett et al. 1979; Aaby and Jacobsen 1979; Hemond 1980; Schurr 1983). This infers that trace metal concentrations in bog waters should be low and less than concentrations in the source water (i.e. precipitation).

There is, however, evidence for metal mobility within peat (Pakarinen and Tolonen 1977; Damman 1978; Norton 1983; Schurr 1983; Urban 1983). High concentrations of dissolved organic carbon (DOC) in bog water (10–70 $mg \cdot L^{-1}$), low pH (3.6–4.5), and alternating oxic/anoxic conditions within peat facilitate dissolution and mobility of metals. Concentrations of Al and Fe greater than those predicted from solubilities of solid phases have been reported for bog waters (Verry 1975; Sparling 1967). This paper reports concentrations of four trace metals (Al, Fe, Zn, and Pb) in bog waters from midcontinental

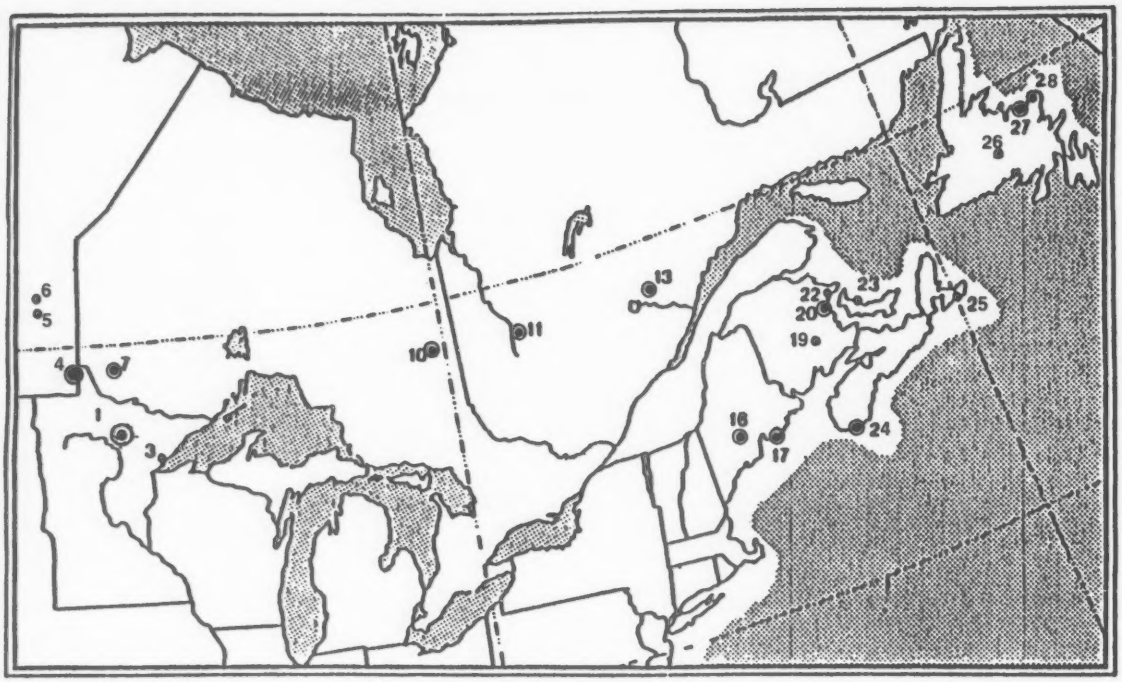

FIG. 1. Bog water samples were collected at 20 sites between Manitoba and the Atlantic seaboard in July and August 1982. Trace metal concentrations for 1982 precipitation (wet-only) were available for sites near bogs 1, 4, 7, 10, 11, and 13 (Chan et al. 1983, 1984; Metzer 1986). Trace metal concentrations in wet-only precipitation near sites 16, 17, 20, 24, and 27 were taken from Munger and Eisenreich (1983), but these were measured prior to 1980.

and eastern North America and examines geographical trends in concentration for evidence of the controlling environmental factors.

Methods

Thirty-seven samples of surface water were collected from 20 bogs along a transect from eastern Manitoba and Minnesota to the Atlantic coast (Fig. 1). Samples were taken from pools and flooded depressions in wetter sites. Where surface water was lacking, sample bottles were pressed into moss surfaces, or pits were dug below the water table and emptied twice before sampling. No differences were found in major-ion chemistry between samples from pools and pits. Samples were filtered in the field through acid-washed glass-fiber filters (1.5-μm pore size) into acid-washed polyethylene bottles and acidified to 1% (vol) with Ultrex® HNO_3. Blanks of distilled, deionized water were treated similarly.

Metals were measured by flameless atomic absorption (Perkin Elmer model 4000 AAS, model 400 HGA). Samples were diluted to 1/10 with distilled, deionized water and analyzed directly for Zn concentrations. To remove organic matter and enhance reproducibility, samples were photooxidized prior to analyses for Al, Fe, and Pb. Samples of 80 mL with 0.4 mL of 30% H_2O_2 added were irradiated for 4 h in quartz tubes with a Hanovia ultraviolet lamp. Field blanks were subjected to the same procedure. Concentrations in the blanks averaged <0.2, 0.3, <8, and <48 $\mu g \cdot L^{-1}$ for Pb, Zn, Al, and Fe, respectively. Spiking prior to oxidation revealed no loss by sorption or precipitation. Precision (SE), based on replicate digestions, was 0.1, 0.6, 2.5, 11.7 $\mu g \cdot L^{-1}$ for Pb, Zn, Al, and Fe, respectively. Detection limits were 0.2, 0.2, 8.0, and 48 $\mu g \cdot L^{-1}$ (Pb, Zn, Al, Fe). Data for pH, absorbance, major ions, and organic carbon have been published for the same set of samples (Gorham et al. 1985).

For 11 of the 20 bogs, concentrations of trace metals in wet-only precipitation at nearby sites were available (Fig. 1). For 6 of these 11 sites, the trace metals in precipitation were measured the same year as the bog water was collected (Chan et al. 1983, 1984; Metzer 1986). For the other five sites (Great Sydney Heath, Bar Harbor, Pt. Sapin, Cape Sable, and Gander Bay), concentrations of trace metals in precipitation were available only for the period 1975–80 (Munger and Eisenreich 1983), and these were interpolated from isopleths rather than measured at sites adjacent to the bogs.

To facilitate comparison with concentrations in bog waters, two methods of correcting trace metal concentrations in precipitation for evapotranspiration were tried. In the first method, the bog water concentration was treated as an annual average and compared with the annual average concentration in precipitation corrected for annual evapotranspiration (ET) (taken as the average of nearby pan evaporation measurements and the calculated Thornthwaite approximation) (Thomas 1953; Ruffner 1978; Canadian Climate Normals 1980; Climate of Southern Ontario 1980; Farnsworth and Thompson 1982). Corrected Concn. = $\text{Concn.}_{\text{rain}}/(1 - \text{ET}/\text{Pptn.})$. (Pptn. = annual precipitation in centimetres). Two biases in this formulation offset each other to some extent. First, mean metal concentrations in summer rain are generally less than the annual average (Chan et al. 1983, 1984; Metzer 1986). Sec-

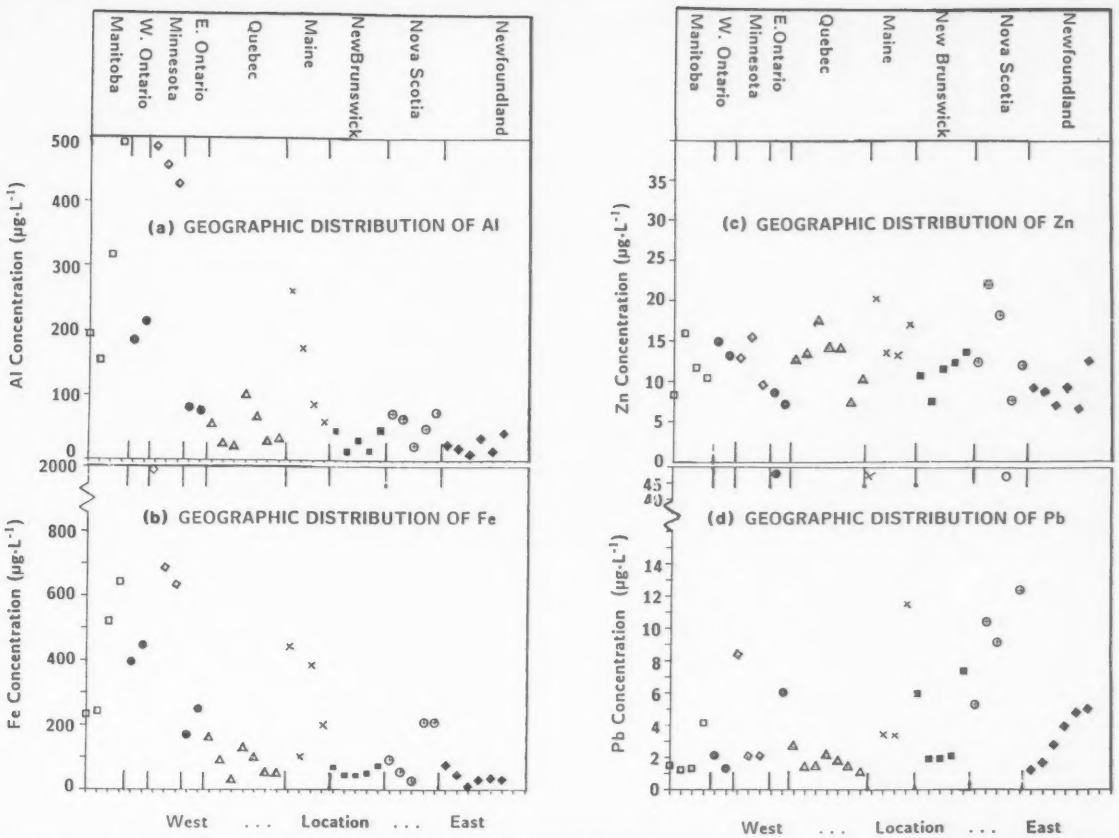

FIG. 2. Geographic distribution of (a) Al, (b) Fe, (c) Zn, and (d) Pb in surface bog waters in North America. Values represent single samples taken in July and August 1982. ◇, Minnesota; □, Manitoba; ●, Ontario; ◇, Quebec; ×, Maine; ■, New Brunswick; ○, Nova Scotia; ◆, Newfoundland.

ond, summertime values of (ET/Pptn.) are higher than the annual average. In the second method, Cl is treated as a conservative tracer to indicate the extent of evaporative concentration. Corr. Concn. = $\text{Concn.}_{\text{rain}} \times (\text{Cl}_{\text{bog}}/\text{Cl}_{\text{rain}})$. Seasonal trends in Cl concentrations are not as pronounced (Munger 1981; Barrie and Sirois 1982; Chan et al. 1983, 1984; Metzer 1986), so the only systematic bias arises from using the annual average metal concentration in precipitation. Thus, bog to rain ratios using this method may be somewhat high.

Results and Discussion

Aluminum

Aluminum concentrations (Fig. 2a) are high, from 154 to 492 $\mu g \cdot L^{-1}$, in seven midcontinental sites (Manitoba, Minnesota, and western Ontario) but, with two exceptions in Maine, are below 103 $\mu g \cdot L^{-1}$ in the 13 sites to the east (eastern Ontario, Quebec, Maine, and the Atlantic Provinces). The high values in Maine both occur in an inland bog (>60 km from the coast) where the ratio of ET to precipitation (0.73) is similar to that of midcontinental bogs (0.61–0.73) and much higher than that of the other eastern sites (0.35–0.55). Thus it is likely that the high values in Maine result from evaporative concentration. Aluminum concentrations measured in this study are comparable with concentrations reported for peatlands in southern Ontario (Sparling 1966) but lower than those found in England (Sparling 1967) or in some midwestern bogs (Verry 1975; Schurr 1983). The high Al concentrations (500–2000 $\mu g \cdot L^{-1}$) reported by Verry (1975) and Schurr (1983) were measured in midsummer during periods of low streamflow when evaporative concentration is greatest.

The gradient of Al concentrations in bog waters, high (279 ± 2 $\mu g \cdot L^{-1}$, geometric mean ± SD) in midcontinental bogs and low in eastern sites (43 ± 2 $\mu g \cdot L^{-1}$), is not caused by the similar gradients in concentration of hydrogen ion (100–200 $\mu eq \cdot L^{-1}$) or DOC (50–70 $mg \cdot L^{-1}$; Gorham et al. 1985). All of the bog waters examined are highly undersaturated with respect to gibbsite and kaolinite. Thus, concentrations of Al are not controlled by pH. Furthermore, although 80–90% of the Al in these bog waters is organically bound (Helmer 1987), complexation is not the factor limiting Al concentrations. The proportion of Al or of (Al + Fe) to DOC is too low to saturate the complexation capacity of the organic matter (Perdue et al. 1976; Johnson et al. 1981; Pott et al. 1985; Helmer 1987). Much higher ratios of Al to DOC are observed in English bogs than in those of North America (Helmer 1987). Thus, neither solubility nor complexation with organic matter is the primary

TABLE 1. Evapotranspiration-corrected ratios of trace metal concentrations in bog waters and precipitation nearby.[a]

Site	Al		Fe		Zn		Pb	
	Pan[b]	Cl[c]	Pan[b]	Cl[c]	Pan[b]	Cl[c]	Pan[b]	Cl[c]
Marcell (Minnesota)	11.7	17.8	39.1	59.6	0.4	0.5	1.9	2.9
Caribou Cluster (Manitoba)								
Mean	3.0	4.3	3.7	5.5	0.7	1.0	0.4	0.5
Min.	2.3	3.4	3.3	4.9	0.7	1.0	0.2	0.3
Max.	3.6	5.3	4.1	6.0	0.8	1.1	0.5	0.8
ELA (Ontario)								
Mean	1.2	1.5	2.3	3.0	0.9	1.1	0.2	0.2
Min.	1.1	1.4	2.2	2.8	0.8	1.0	0.2	0.2
Max.	1.3	1.6	2.5	3.2	0.9	1.2	0.2	0.3
Diamond (Ontario)								
Mean	1.5	2.6	3.1	5.3	0.6	1.0	3.3	5.6
Min.	1.5	2.5	2.5	4.2	0.5	0.9	0.7	1.2
Max.	1.6	2.7	3.7	6.3	0.6	1.1	5.9	10
Lac Parent (Quebec)								
Mean	0.7	1.0	1.6	2.4	0.9	1.3	02	0.3
Min.	0.5	0.7	1.2	1.7	0.9	1.3	0.1	0.2
Max.	1.0	1.4	2.1	3.0	0.9	1.4	0.2	0.4
Lac St. Jean (Quebec)								
Mean	0.8	0.3	0.8	0.3	0.8	0.3	0.1	0.04
Min.	0.3	0.1	0.4	0.1	0.5	0.2	0.1	0.03
Max.	1.5	0.6	1.5	0.6	1.1	0.4	0.1	0.05
Gt. Sydney Heath (Maine)								
Mean			0.9	4.0	0.1	0.4	0.4	1.7
Min.			0.4	1.5	0.1	0.3	0.1	0.2
Max.			1.5	6.6	0.1	0.4	0.7	3.2
Bar Harbor (Maine)								
Mean			6.0	2.1	0.2	0.1	0.4	0.1
Min.			4.1	1.4	0.2	0.1	0.2	0.1
Max.			8.0	2.8	0.2	0.1	0.6	0.2
Pt. Sapin (New Brunswick)								
Mean			0.7	0.2	0.1	0.04	0.2	0.1
Min.			0.5	0.2	0.1	0.04	0.1	0.1
Max.			0.8	0.3	0.2	0.05	0.4	0.1
Cabe Sable (Nova Scotia)			0.7	0.2			0.6	0.2
Gander Bay (Newfoundland)								
Mean							0.3	0.1
Min.							0.3	0.1
Max.							0.3	0.2
Mean	3.2	4.6	5.9	8.2	0.5	0.6	0.5	1.1

[a]Rain concentrations from Metzer (1986), Chan et al. (1983, 1984), Munger and Eisenreich (1983), and Barrie and Sirois (1982). No precipitation data were available for sites near to bog sites. Means represent two to five bog water samples.
[b]Rain concentrations corrected for ET using mean of pan and Thornthewaite values of ET.
[c]Rain concentrations corrected for ET using the ratio of Cl in rain and bog waters.

determinant of Al concentrations in bog waters.

The gradient of Al concentrations in bog waters also mirrors the gradient in atmospheric deposition of Al (Thornton and Eisenreich 1982; Chan et al. 1983, 1984; Barrie and Vet 1984; Metzer 1986). Similar concentration gradients are observed for Fe, Ca, and K in bog waters (Gorham et al. 1985). The chemistry and biological uptake of these four elements differ markedly in bogs (e.g. Damman 1978; Schurr 1983). Therefore, it is likely that the common origin of these four elements, atmospheric deposition of soil dust, is responsible for their

concentration gradients in bog waters across northeastern North America.

The relative importance of sources other than wet deposition (e.g. dry deposition, release from peat) and sinks (sorption to peat, plant uptake, mineral formation) for trace metals in bogs may be seen from a comparison of metal concentrations in precipitation and bog waters from nearby sites (Table 1). Evapotranspiration increases concentrations in bog water above those supplied in precipitation and reinforces the geographic trend in Al deposition because ET is highest relative to precipitation in the midcontinent. Both methods of correcting precipitation concentrations for ET (Table 1) yield the same conclusions: additional sources of Al must be larger than all sinks for Al in Minnesota and Manitoba bogs, but such sources decline in importance to the east. Dry deposition (1 to 2 times wet deposition) and release of Al from deeper, mineral-rich layers of peat contribute to the high ratios at the Marcell, Minnesota, site (Urban 1987). Dry deposition is probably an important source of Al in the Manitoba bog as well due to its proximity to the agricultural prairie region. Ratios below 1 in the Quebec bogs suggest that sinks for Al, probably sorption to peat, are larger than dry deposition and release of Al from peat. Such sinks are likely to occur in all bogs but can only be seen by this type of analysis when they exceed nonprecipitation sources.

Iron

Concentrations of Fe in bog water range from 233 to 1820 $\mu g \cdot L^{-1}$ in the midcontinent and, again with two exceptions in Maine, from 12 to 251 $\mu g \cdot L^{-1}$ eastward (Fig. 2b). Unlike Al, high concentrations of Fe are found in both the coastal and inland bogs in Maine. As for Al, high rates of ET may be responsible for the high concentrations in the inland bog in Maine where ratios of Fe in bog water to Fe in rain are near unity when the latter are corrected for ET (Table 1). Reasons for high concentrations in the Bar Harbor site are unknown. The high value (1820 $\mu g \cdot L^{-1}$) in the Marcell bog is not atypical of this site. Over a 4-yr period, summertime concentrations ranged from 1000 to 2150 $\mu g \cdot L^{-1}$ at this site. These high concentrations reflect the high summer ET rate, release from deeper minerotrophic peat, and perhaps proximity (50 km) to active Fe mining.

Iron is similar to Al in its geographical trend and relationship to agricultural dustfall. The geometric mean concentration for midcontinental sites is 442 $\pm$ 2 $\mu g \cdot L^{-1}$ but is only 80 $\pm$ 2 $\mu g \cdot L^{-1}$ for eastern sites. In midcontinental bog waters, Fe concentrations are higher than concentration in precipitation adjusted for ET (Table 1). As for Al, additional sources (dry deposition and release from peat) are greater than sinks for Fe (sorption to peat, precipitation of sulfides or other minerals) in this region. In at least three of the bogs to the east, low ratios (bog to rain) indicate that sorption by peat is greater than dry deposition and release from peat.

Zinc

Zinc exhibits a nearly constant concentration (range = 7–22 $\mu g \cdot L^{-1}$, geometric mean = 13 $\pm$ 1.4 $\mu g \cdot L^{-1}$) in North American bog waters. Concentrations in bog waters are, on average, between 52 and 60% of concentrations in precipitation (adjusted for ET). Sinks (sorption to peat, plant uptake) must therefore be greater than additional sources of zinc (dry deposition) throughout this region.

Lead

Concentrations of Pb are low (1.1–12.4 $\mu g \cdot L^{-1}$) across the study region except in three widely separated sites where concentrations in one of two or three samples are between 40 and 50 $\mu g \cdot L^{-1}$. None of the three sites is near a major point-source of Pb, although none is far from urban centers or smelters. Lead concentrations are especially low (1.4–4.2 $\mu g \cdot L^{-1}$) in the midcontinental bogs that are westward of major emission sources.

Lead is bound by peat to a great extent. Concentrations of Pb in bog waters are less than 60% of adjusted concentrations in precipitation in 7 of 11 sites (Table 1). With the exception of the precipitation Pb values for New Brunswick, Nova Scotia, and Newfoundland, all precipitation measurements were taken in 1982, the year of bog water sampling, so comparisons are valid despite the rapidly changing atmospheric burden of Pb (Eisenreich et al. 1986). Concentrations of Pb in precipitation over the Maritime Provinces were taken from Munger and Eisenreich (1983) and are from an earlier time period. These may be 25–45% higher than concentrations in 1982 due to the decrease in use of leaded gasoline in Canada (Peter Harris, Environment Canada, pers. comm.). If dry deposition of Pb is negligible, the range of bog to rain ratios indicates that from 4 to 60% of Pb deposited in bogs by wet deposition (excluding high values from Diamond, Marcell, and Great Sydney Heath bogs) may be lost in runoff from peatlands. This variability in retention of atmospheric inputs may restrict the utility of regional comparisons of Pb concentrations in peat as an indication of atmospheric deposition. This mobility should be examined further to justify the validity of Pb-210 dating in peat.

Soil Enrichment Factors

The role of soils as the source of elements and the relative mobilities of elements in bog waters may be evaluated by calculating enrichment factors. The enrichment factor (EF) is defined as the ratio of any element (x) in bog water, normalized to the concentration of Al (an element thought to be supplied primarily from soil dust), to the concentration of that element in soil, again normalized to Al (Rahn 1976):

$$EF = (x_{bog}/Al_{bog})/(x_{soil}/Al_{soil}).$$

If soil is the sole source of an element in bog water and the element is dissolved to the same extent as Al, the EF will be 1. Differences in solubility between Al and other elements will result in somewhat higher or lower EF's. However, if soil is the major source of an element, its EF will not change appreciably with changing concentrations of Al in bog water. In contrast, elements that are supplied mainly from nonsoil sources will exhibit declining EF's with increasing concentrations of Al in bog water. Enrichment factors for Fe, Zn, and Pb were calculated using the mean composition of crustal rock (Bowen 1979). Enrichment factors are plotted versus Al concentrations in bog waters in Fig. 3.

Iron is enriched up to 10-fold over Al in bog waters (Fig. 3a). The EF does not vary with increasing Al concentration, but rather the elements themselves co-vary. It is likely, therefore, that both are supplied primarily by atmospheric deposition of soil particles. Slight enrichment of Fe in aerosols (Rahn 1976) and precipitation (Metzer 1986) has been noted previously and ascribed to soil–aerosol fractionation. The higher bog to rain ratios of Fe compared with Al at sites from Minnesota to Quebec indicate that Fe may be solubilized to a

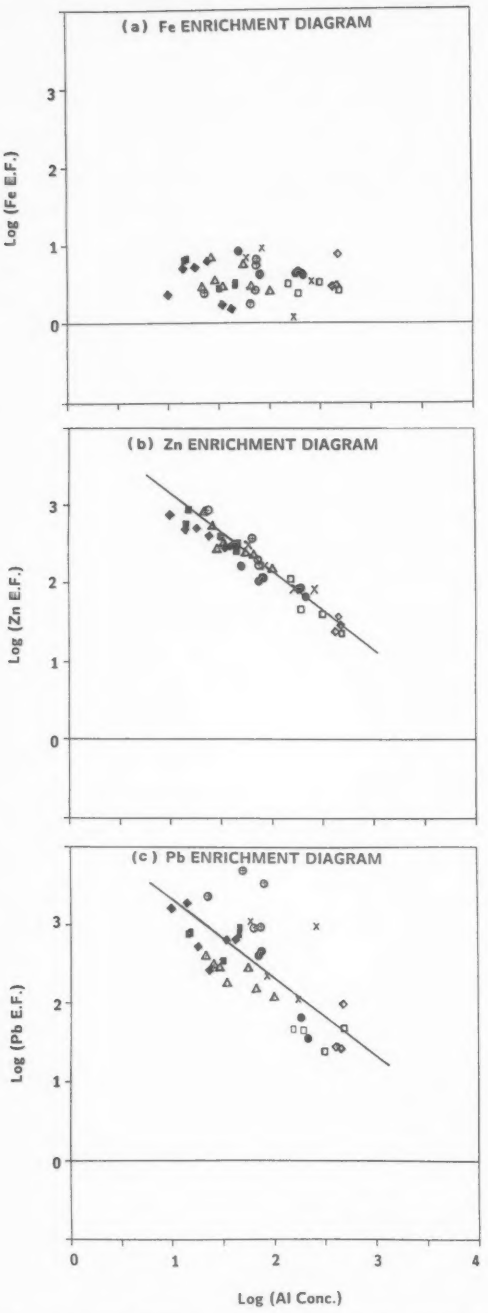

FIG. 3. Enrichment factor diagrams for (a) Fe, (b) Zn, and (c) Pb. Fe is slightly enriched relative to Al and co-varies with Al. Zn and Pb are highly enriched relative to Al. Concentrations of Pb and Zn are independent of Al concentrations. Locations symbolized as in Fig. 2.

slightly greater extent than Al in bog waters. A higher affinity of dissolved organics for Fe than for Al may be responsible for enhanced dissolution (and hence the enrichment in Fe in bog waters) and might also explain the closer correlation between DOC and Fe than between DOC and Al in Nova Scotian lakes receiving bog drainage (E. Gorham, J. K. Underwood, F. B. Martin, and J. G. Ogden, unpubl. data). Enrichment factors for Fe in low-DOC bog waters from England (N. R. Urban, unpubl. data) are similar to EF values for Fe in North American bog waters. This suggests that interactions with dissolved organic matter are of secondary importance and atmospheric deposition is the primary control on concentrations of Fe and Al in bog waters.

Despite sorption to peat of approximately half of wet-depositional inputs (Table 1), Zn is strongly enriched (up to nearly 1000-fold) over Al in bog waters (Fig. 3b), particularly in the eastern bogs lowest in Al concentration and downwind of major sources of air pollution (smelters and urban industrial areas). The high EF's are not caused by a greater mobility of Zn relative to Al in bogs (Table 1), but by anthropogenic enrichment of Zn in atmospheric particles and deposition (Rahn 1976; Metzer 1986). The diagonal declining at 45° through the geometric mean represents a constant Zn concentration. Location of the bog water data set along that line indicates that Zn does not co-vary with Al, contrary to the case for Fe.

Lead is also strongly enriched in bog waters (Fig. 3c); EF's range nearly to 6000. As in the case of Zn, EF's are highest in eastern bog waters, low in Al and downwind of major sources of air pollution, and lie along a declining 45° diagonal indicating no relationship of Pb to Al. (The three waters with abnormally high Pb concentrations lie well above on a parallel diagonal.) As with Zn, sorption by peat of a major fraction of Pb inputs lowers the Pb EF in bog waters relative to EF's in precipitation and aerosols (Rahn 1976; Metzer 1986).

Summary and Conclusions

Examination of trace metal concentrations in bog waters along a transect across northeastern North America revealed large gradients in concentrations of Fe and Al (increasing east to west) and more nearly constant concentrations of Pb and Zn. Important variables controlling trace metal concentrations include the magnitude of wet and dry atmospheric deposition, the ratio of ET to precipitation, release of metals from peat, and sorption of trace metals by peat. A comparison of concentrations of Fe and Al in bog waters and in precipitation showed that significant sources other than wet deposition exist in midcontinental bogs. Such sources (dry deposition and release from peat) decline in importance eastward. Atmospheric deposition of soil dust appears to be the major source and control of Fe and Al concentrations in bog waters. Concentrations of Pb and Zn in bog waters are less than concentrations in precipitation (adjusted for ET). Thus, removal mechanisms (sorption to peat) are important in controlling concentrations of Pb and Zn in bog waters. Enrichment factors indicate that Fe in bog waters comes primarily from soil dustfall and that Pb and Zn, particularly in bogs east of Ontario, are primarily of anthropogenic origin. Thus, bog water composition can be used to indicate sources and magnitudes of element inputs as well as processes occurring within bogs.

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Influence of Complexation and pH on Individual and Combined Heavy Metal Toxicity to a Freshwater Green Alga

M. E. Starodub and P. T. S. Wong

Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

C. I. Mayfield

Department of Biology, University of Waterloo, Waterloo, Ont. N2L 3G1

and Y. K. Chau

Department of Environment, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

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The effect of complexation and pH on heavy metal (Cu, Zn, Pb) toxicity to a freshwater green alga, *Scenedesmus quadricauda*, was investigated. Extracellular ligands produced by *S. quadricauda* were capable of binding heavy metals and reducing their single and combined toxicities. Apparent complexing capacities and the ability of the sediment humics and artificial complexing agents such as ethylenediaminetetraacetic acid (EDTA), citric acid, and glycolic acid to ameliorate Cu, Zn, or Pb toxicity were also assessed. The toxicity of metals to algal growth was enhanced at acidic pH. Combined toxicity of these metals was significantly greater at pH 4.5 than at pH 8.5 or pH 6.5. Synergistic effects (between Cu, Zn, and Pb) towards algal growth increased at low pH. Specific heavy metals, their respective concentrations, the presence of complexing ligands, and pH influence both individual and combined heavy metal toxicities.

Les auteurs ont étudié l'incidence de la complexation et du pH sur la toxicité de métaux lourds (Cu, Zn, Pb) pour *Scenedesmus quadricauda*, une algue verte dulçaquicole. Des ligands extracellulaires produits par *S. quadricauda* étaient capables de fixer des métaux lourds et de réduire ainsi leur toxicité simple et combinée. Ils ont aussi évalué les capacités apparentes de complexation et la faculté des matières humiques des sédiments et des agents artificiels de complexation comme l'acide éthylènediaminetétracétique (EDTA), l'acide citrique et l'acide glycolique pour ce qui est de l'amélioration de la toxicité du Cu, du Zn et du Pb. À des pH acides, la toxicité des métaux pour la croissance algale a été augmentée tandis que la toxicité combinée de ces métaux était nettement plus élevée au pH 4,5 qu'au pH 8,5 ou 6,5. Les incidences synergiques (entre Cu, Zn et Pb) sur la croissance algale ont augmenté à un faible pH. Certains métaux lourds, leurs concentrations, la présence de ligands de complexation et le pH influent sur les toxicités individuelle et combinée des métaux lourds.

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Mobility, bioavailability, and toxicity of metals are dependent on their physico-chemical forms in the aquatic environment (Alberts and Giesy 1983; Giesy 1983). Metal availability and toxicity to the aquatic biota are influenced by binding to organics, precipitation, and ionic interactions. Organic complexing agents in natural waters, although generally not as abundant as inorganic ligands, possess a strong affinity for heavy metals, especially Cu (Borgmann 1983). Stevenson and Ardakani (1972) and Stiff (1971) found that most metals are transported in natural waters as organic complexes. Hodson et al. (1979) suggested that chelation is the single most important abiotic factor in the reduction of Cu toxicity in aquatic ecosystems. Many studies have revealed that both natural and synthetic chelators or metal–organic complexes alter the toxicity of metal ions to aquatic organisms (Khobot'yer et al. 1975; Bitton and Freihofer 1978; Gadd and Griffiths 1978; Gnassia-Barelli et al. 1978; Van den Berg et al. 1979). Metal–organic complexes reduce the bioavailability and toxicity of heavy metals, probably by decreasing the attraction between the chelated metals and the negative surface of the cell (Babich and Stotzky 1983). Although binding of metals to organics or solids usually reduces metal toxicity, toxicity has been known to persist even when there are no free metallic ions present (Gadd and Griffiths 1978).

Increased toxicity of metals to algae has been observed at alkaline pH (Steemann Nielsen and Kamp-Nielsen 1970; Gachter 1976; Hargreaves and Whitton 1976a, 1976b; Peterson et al. 1984). Other authors have reported a general decrease in metal toxicity at high pH (Gachter 1976; Monahan 1976; Steemann Nielsen and Bruun Laursen 1976; Harding and Whitton 1977; Rai et al. 1981).

Speciation of metals in aqueous solution influences their chemical behaviour. Studies of the influence of abiotic factors on toxicities of multiple toxicants, including heavy metals, are virtually nonexistent. Measurements of the complexing capacities of natural (algal exudates, sediment humics) and artificial (ethylenediaminetetraacetic acid (EDTA), citric acid, glycolic acid) complexing agents and an assessment of the effect of these complexing agents on individual and combined heavy metal toxicity are presented in this paper. Reports of different effects of pH prompted the present studies of the response of a freshwater green alga, *Scenedesmus quadricauda*, to both individual and combined metal exposures over a range of pH.

Materials and Methods

Test Alga and Growth Conditions

The freshwater green alga *S. quadricauda* was grown in a modified CHU-10 medium (pH 8.0) (Wong et al. 1982) containing no complexing agents at 20°C on a rotary shaker (100 rpm) under conditions of 18 h light (108 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 6 h darkness until cells reached the logarithmic phase of growth. These standard incubation conditions of temperature, light, and agitation were maintained during all assays for metal toxicity. Algal cell densities were determined by direct microscopic counts with a Zeiss microscope at 200× magnification in a Fuchs Rosenthal counting chamber. A standard inoculum of 2.0×10^5 cells$\cdot mL^{-1}$ (in log phase) was used for all subsequent algal bioassays. Metal solutions ($CuSO_4$, $ZnSO_4$, or $PbCl_2$) were prepared by dissolving reagent grade metal compounds in doubly distilled water.

Collection of Algal Exudates

Batch cultures of *S. quadricauda* were prepared by inoculating glass carboys containing 8 L of CHU-10 (pH 8.0) with logarithmic cells to yield an initial population density of approximately 8.0×10^3 cells$\cdot mL^{-1}$. Algae were grown phototrophically at 20°C with agitation, aeration, and constant illumination (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) until senescence. Total incubation period was 4–5 wk. Algal exudates from each 8-L carboy were collected by pressure filtration through a 0.45-μm membrane filter (Millipore HA filter, 140-mm diameter) and were then concentrated on a rotary evaporator at 50°C to a final volume of 1 L. This temperature was used to avoid denaturation of heat-labile organics present in the algal exudates. Concentrated exudates were frozen until the contents of ten 8-L carboys had been filtered and concentrated. The concentrated exudates were thawed and pooled. Chloride analysis of pooled concentrated algal exudates with a modified mercuric thiocyanate method (Environment Canada 1974) revealed an excessive ion concentration; therefore, they were dialysed in cellulose dialysis tubing (molecular weight 1000). Desalting of the exudates via gel filtration, Sephadex G-25, or anion exchange resins was attempted, but proved unsatisfactory due to separation of various carbohydrates and simultaneous elution of the carbohydrate fractions with the chloride indicator ion. Active complexing algal exudates were estimated as micrograms per millilitre dextrose equivalent by the phenol/sulphuric acid method of Dubois et al. (1956) and protein as micrograms per millilitre bovine serum albumin equivalent by the Lowry method (Lowry et al. 1951).

Collection of Sediment Elutriate

Preparation of Hamilton Harbour sediment elutriate was based on the method described in U.S. Environmental Protection Agency (1973). One part Hamilton Harbour sediment (160 g) was combined with four parts double-distilled water (640 mL) in a 1-L Erlenmeyer flask. Air bubbling through a deionized water trap was used to aerate the sediment/water mixture for 24 h. During this period, the reaction flask was periodically shaken to facilitate a homogeneous suspension. After the 24-h elutriation period, aeration was stopped and the sediment suspension allowed to settle for no more than 1 h. The elutriate was decanted and centrifuged at 6000 × *g* for 20 min. Filtration of the supernatant through a Whatman No. 4 qualitative paper, followed by a further filtration through a 0.45-μm Millipore HA filter, concluded the collection of the Hamilton Harbour sediment elutriate. Carbohydrate content was determined with the phenol/sulphuric acid method (Dubois et al. 1956).

Measurement of Complexing Capacities

Prior to the determination of complexing capacity, nonpooled concentrated algal exudates, nondialysed pooled concentrated algal exudates, dialysed pooled algal excudates, Hamilton Harbour sediment elutriate, 1 μ*M* EDTA, 1 μ*M* citric acid, and 1 μ*M* glycolic acid were filtered under mild vacuum through a 0.45-μm Millipore HA filter to remove particles. Complexing capacities of the natural and artificial chelators were determined by differential pulse anodic stripping voltammetry (DPASV) according to the method of Chau et al. (1974). Aliquots of these chelators were titrated with various amounts of Cu^{2+} and allowed to equilibrate for at least 1 h at constant room temperature. An acetate buffer (pH 4.6) prepared from glacial acetic acid (suprapuregrade, Merck-BDH) and anhydrous sodium acetate (suprapuregrade, Merck-BDH) was used to buffer titrated samples and the free Cu^{2+} concentration in each was measured by DPASV with a total plating period of 2 min. From a plot of peak current (microamperes) versus Cu (micrograms per litre) spike, the complexing capacity (*x*-intercept) of the respective chelator was determined. Complexing capacities were recorded as micromolar Cu^{2+} equivalent. The complexation of Zn and Pb individually by nondialysed pooled algal exudates was also determined by titration of nondialysed pooled algal exudates with known amounts of the respective metal. Nondialysed and dialysed pooled algal exudates were titrated with known amounts of Cu, Zn, and Pb in combination, equilibrated, and the respective free metal ion concentrations measured by DPASV.

Algal Bioassays

Preliminary assays were performed to determine the concentration of each metal that caused a 50% reduction in photosynthetic activity (EC_{50}) determined by probit analysis (Finney 1971). Erlenmeyer flasks (25 mL) containing CHU-10 (pH 8.0) and increasing concentrations of single metals were inoculated with 1.0 mL of standard inoculum. The final volume per flask was 15 mL. Flasks were placed in a random order on the shaker and incubated under standard conditions of temperature, light, and agitation for 20 h. After 20 h, 0.1 mL of ^{14}C-labeled sodium bicarbonate (7.4×10^4 Bq$\cdot mL^{-1}$) was added to each flask and flasks were incubated a further 4 h.

TABLE 1. Measured complexing capacities for both natural and artificial complexing agents.

Complexing agent	Complexing capacity (μM Cu^{2+} equiv.)	CHO ($\mu g \cdot mL$ dextrose^{-1})	pH	CL ($\mu g \cdot L^{-1}$)
EDTA ($1\ \mu M$)	0.90	—	—	—
Citric acid ($1\ \mu M$)	ND[a]	—	—	—
Glycolic Acid ($1\ \mu M$)	ND[a]	—	—	—
CHU-10	ND[a]	ND[b]	8.00	20
Nondialysed nonpooled concentrated algal exudates	0.12	8.20	—	—
Dialysed pooled concentrated algal exudates	0.34	4.60	7.00	14
Nondialysed pooled concentrated algal exudates	0.71	12.45	7.90	144
Hamilton Harbour sediment elutriate	1.50	60.00	—	—

[a]Not detectable by DPASV method of Chau et al. (1974).
[b]Not detectable by the method of Dubois et al. (1956).

Light controls (no metals) were also prepared. All samples were prepared in triplicate. Photosynthetic activity was stopped at exactly 4 h and ^{14}C uptake per flask was determined according to the method of Wong et al. (1982).

To determine the ability of the synthetic chelators EDTA, citric acid, and glycolic acid to ameliorate metal toxicity, the above procedure was repeated with the following exceptions. EDTA, citric acid, or glycolic acid was added to flasks containing CHU-10 (pH 8.0) to give a final concentration of 2 μM for each complexing agent. Copper from 0 to 1000 $\mu g \cdot L^{-1}$ was added to sets of flasks containing either 2 μM EDTA, citric acid, or glycolic acid, which were then autoclaved and the remainder of the bioassay carried out as described earlier. This assay was repeated replacing Cu with Pb (0–5000 $\mu g \cdot L^{-1}$) and finally with Zn (0–1000 $\mu g \cdot L^{-1}$). The EC_{50} of each metal in the presence of the respective complexing agent was calculated.

The ability of dialysed pooled concentrated algal exudates and Hamilton Harbour sediment elutriate to ameliorate the toxicity of Cu, Zn, and Pb singly and in combination to algal photosynthetic activity was investigated. Metal concentrations examined were Cu at 0, 100, and 200 $\mu g \cdot L^{-1}$, Zn at 0, 300, and 500 $\mu g \cdot L^{-1}$, and Pb at 0, 3000, and 6000 $\mu g \cdot L^{-1}$. These concentrations were chosen to study based on the EC_{50} values for metals determined in the absence of complexing agents. The organic nature of these natural chelators may be altered by high temperatures; therefore, it was necessary to autoclave flasks containing CHU-10 (pH 8.0) and metals prior to their addition. Chelators (1.0 mL of dialysed pooled concentrated algal exudates or 0.1 mL of Hamilton Harbour sediment elutriate) were added to cooled flasks and flasks were held at room temperature for 30 min to allow metals and chelators to equilibrate and form complexes before receiving the standard inoculum. The final volume per flask was 15 mL. Flasks were incubated under standard conditions of temperature, light, and agitation for 20 h, after which ^{14}C uptake over the following 4 h was determined as described previously.

pH and Metal Toxicity

Exposure of *S. quadricauda* to Cu, Zn, and Pb (singly and in combination) at pH 4.5, 6.5, and 8.5 was investigated. Metal concentrations examined were Cu at 0, 50, 100, and 200 $\mu g \cdot L^{-1}$, Zn at 0, 100, 225, and 500 $\mu g \cdot L^{-1}$, and Pb at 0, 1000, 3000, and 6000 $\mu g \cdot L^{-1}$. Pyrex tubes in duplicate (final volume 15.0 mL) containing respective metal concentrations in CHU-10 at each pH received 1.0 mL of standard inoculum. The tubes were incubated at 20°C under continuous illumination (108 $\mu E \cdot m^{-2} \cdot s^{-1}$) for a 15-d period, at the end of which the pH of each tube was measured. Growth was monitored daily by optical density at 665 nm (Bausch and Lomb Spectronic 20). These readings were converted into average growth rates according to the equation $\mu_{avg} = (\ln X(T) - \ln X_0)/T$ (Nyholm 1985) where μ_{avg} = average algal growth rate per day, $X(T)$ = biomass concentration at time T, T = time in days, and X_0 = inoculated biomass concentration (at time zero).

Results and Discussion

Complexation

Characteristics of complexing agents, both natural and artificial are listed in Table 1. Protein content of algal exudates were $<2.5\ \mu g \cdot mL^{-1}$, below the detection limits of the Lowry method (Lowry et al. 1951).

Previous work of Chau and Wong (1975) revealed that natural waters low in organic carbon have little to no complexing capacity. Complexing capacities of the natural

TABLE 2. Complexation of Cu, Zn, and Pb singly and combined by nondialysed and dialysed pooled concentrated algal exudates (— = not determined).

Complexing agent	CHO ($\mu g \cdot mL$ dextrose^{-1})	Complexing capacity (μM)			Metals combined (μM)	Metal complexed (μM)
		Cu^{2+}	Zn^{2+}	Pb^{2+}		
Nondialysed algal exudates	12.45	0.71	0.50	0.10	0.5 Cu, 0.5 Zn, and 0.1 Pb	0.50 Cu 0.48 Zn 0.06 Pb
					1.50 Cu, 1.50 Zn, and 0.30 Pb	1.00 Cu 1.46 Zn 0.00 Pb
Dialysed algal exudates	4.66	0.21	—	—	1.0 Cu, 1.0 Zn, 0.1 Pb	0.53 Cu 0.75 Zn 0.06 Pb

TABLE 3. Effect of various complexing agents on the toxicity of Cu, Zn, and Pb to the photosynthesis of *S. quadricauda*.

Metal	EC_{50} ($\mu g \cdot L^{-1}$)[a]			
	No complexing agent	EDTA (2 μM)	Citric acid (2 μM)	Glycolic acid (2 μM)
Cu	100	300	135	90
Zn	250	250	290	210
Pb	2700	NT[b]	3200	NT[b]

[a]EC_{50} = concentration causing a 50% reduction in photosynthesis after a 4-h incubation.
[b]No toxic effect observed.

chelators, algal exudates, and Hamilton Harbour sediment elutriate increased with increased carbohydrate content (Table 1). The observed increase in complexing capacity, however, was not proportional to the increased carbohydrate content. Protein content of the algal exudate was <2.5 $\mu g \cdot mL^{-1}$ and probably did not contribute to this observation. Amide groups present in low amounts could have contributed to the complexing capacity of the algal exudates. Complexing capacities of the algal exudates for Cu, Zn, and Pb reveal that Cu was the most strongly complexed metal. However, when Cu, Zn, and Pb were allowed to equilibrate simultaneously with the same exudates, Zn was preferentially bound, followed by Cu and then Pb (Table 2). Tnis apparent selective binding of Zn may be related to its requirement as an essential nutrient and could have significantly affected the observed multiple heavy metal toxicity to *S. quadricauda*.

Metal toxicities to the photosynthetic activity of *S. quadricauda* were influenced by the addition of complexing agents to the growth medium (Table 3). In the absence of a complexing agent, Cu was the most toxic with an EC_{50} of 100 $\mu g \cdot L^{-1}$, followed by Zn at 250 $\mu g \cdot L^{-1}$, while Pb was relatively less toxic with an EC_{50} value of 2700 $\mu g \cdot L^{-1}$ (Table 3). Citric and glycolic acids form weak and labile complexes with Cu and had no significant ameliorative effect on Cu toxicity to *S. quadricauda* (Tables 1 and 3). Each acid did offer significant protection against Pb toxicity but not against the toxic effect of Zn to algal photosynthetic activity. Glycolic acid (2 μM) slightly enhanced Zn toxicity (Table 3). Complexes formed between glycolic acid and Zn may have enhanced algal uptake of this metal, allowing higher concentrations of Zn to penetrate the cell. In contrast, EDTA, a synthetic complexing agent and contaminant of natural waters, demonstrated a strong complexing capacity for Cu (Table 1). In the presence of 2 μM EDTA, the order of metal toxicity to *S. quadricauda* was Zn > Cu >> Pb. EDTA (2 μM) significantly ameliorated Cu and Pb toxicity (Table 3).

Algal cells by releasing extracellular products may control the bioavailability of nutrients and metals (i.e. enhance uptake of nutrients, or prevent uptake of metals) in their immediate environment (Van den Berg et al. 1979; Rai et al. 1981). Algal photosynthetic activity was inhibited by approximately 50–58% upon exposure to Cu (100 and 200 $\mu g \cdot L^{-1}$), Zn (250 and 500 $\mu g \cdot L^{-1}$), or Pb (3000 and 6000 $\mu g \cdot L^{-1}$). Combinations of two or three metals also significantly inhibited ^{14}C uptake by *S. quadricauda* (Table 4). In the presence of algal exudates the toxic effects of both single metals and metal mixtures were ameliorated (Table 4).

An elutriate prepared from Hamilton Harbour sediment had a high carbohydrate content and demonstrated a strong complexing capacity (Table 1). Unlike the algal exudates, the addition of sediment elutriate had less protective effect against metal toxicity (Table 5). While the presence of 0.1 mL of elutriate significantly reduced toxicities of Cu (100 $\mu g \cdot L^{-1}$) and Zn (500 $\mu g \cdot L^{-1}$), the individual toxicities of Pb (3000 and 6000 $\mu g \cdot L^{-1}$), Cu (200 $\mu g \cdot L^{-1}$), and Zn (250 $\mu g \cdot L^{-1}$) were only slightly reduced (Table 5). No significant reduction in toxicity was observed for any combinations of Cu, Zn, and Pb examined. The sediment elutriate was slightly inhibitory to photosynthesis. The elutriate probably contained inorganic and organic pollutants, due to heavy industrial use of the water, that may counteract its protective complexing property.

Many methods are available for describing the complex interactions between toxic chemicals and organisms (Borgmann 1980; Marking 1985). In the present study, three methods were used to analyse our results obtained in Tables 4 and 5. The three methods used were $P_{cal} = P_x P_y P_z$ (Colby 1967) and $[(1/P_{cal}) - 1] = [(1/P_x) - 1] + [(1/P_y) - 1] + [(1/P_z) - 1]$ (Borgmann 1980), where P_{cal} = calculated response and $P_{x,y,z}$ = observed response for individual toxicants, and $y = a_x + b_y$ (Voyer and Heltshe 1984), where $y =$

TABLE 4. Amelioration of single and combined heavy metal effects on photosynthesis by dialysed pooled concentrated algal exudates (1.0 mL). *Significantly different from the control[a] (Student *t*-test, $P = 95\%$, df = 5); **Significantly different from the control[b] (Student *t*-test, $P = 95\%$, df = 5).

Metal ($\mu g \cdot L^{-1}$)			No complexing agents (% control[a] ± SD, ($N = 3$)	Dialysed algal exudates (% control[b] ± SD, ($N = 3$)
Cu	Zn	Pb		
0[c]	0[c]	0[c]	100 ± 10	100 ± 20
100	0	0	50 ± 1*	77 ± 7
200	0	0	42 ± 2*	53 ± 4**
0	250	0	44 ± 7*	72 ± 1
0	500	0	49 ± 5*	95 ± 4
0	0	3000	47 ± 2	112 ± 4
0	0	6000	51 ± 5	139 ± 28
100	0	3000	66 ± 6*	87 ± 15
200	0	6000	82 ± 3	87 ± 11
100	0	6000	86 ± 1	151 ± 17**
200	0	3000	88 ± 1	97 ± 4
100	250	0	59 ± 3*	81 ± 5
200	500	0	49 ± 3*	92 ± 0
100	500	0	57 ± 4*	86 ± 9
200	250	0	52 ± 5*	81 ± 8
0	250	3000	42 ± 5*	78 ± 7
0	500	6000	71 ± 1*	112 ± 3
0	500	3000	55 ± 6*	98 ± 10
0	250	6000	56 ± 2*	86 ± 13
100	250	3000	59 ± 7*	78 ± 10
200	250	3000	53 ± 3*	77 ± 1
100	250	6000	49 ± 4*	90 ± 6
200	250	6000	48 ± 5*	76 ± 4
100	500	3000	55 ± 5*	80 ± 5
200	500	3000	48 ± 4*	74 ± 1
100	500	6000	61 ± 5*	96 ± 5
200	500	6000	52 ± 2*	77 ± 3

[a] ^{14}C uptake is presented as % control without complexing agents; 100% = 29 428 dpm.
[b] ^{14}C uptake is presented as % control with dialysed algal exudates; 100% = 20 894 dpm.
[c] Control.

TABLE 5. Amelioration of single and combined heavy metal effects on photosynthesis by Hamilton Harbour sediment elutriate (0.1 mL). *Significantly different from the control[a] (Student *t*-test, $P = 95\%$, df = 5); **Significantly different from the control[b] (Student *t*-test, $P = 95\%$, df = 5).

Metal ($\mu g \cdot L^{-1}$)			No complexing agents (% control[a] ± SD, ($N = 3$)	Dialysed algal exudates (% control[b] ± SD, ($N = 3$)
Cu	Zn	Pb		
0[c]	0[c]	0[c]	20 ± 10	100 ± 15
100	0	0	61 ± 9*	83 ± 20
200	0	0	53 ± 4*	58 ± 11**
0	250	0	42 ± 2*	56 ± 10**
0	500	0	55 ± 6*	75 ± 5
0	0	3000	49 ± 6*	67 ± 8**
0	0	6000	58 ± 5*	68 ± 5**
100	0	3000	55 ± 1*	65 ± 5**
200	0	6000	51 ± 5*	59 ± 8**
100	0	6000	57 ± 6*	75 ± 7
200	0	3000	47 ± 2*	53 ± 2**
100	250	0	50 ± 4*	61 ± 6**
200	500	0	50 ± 2*	63 ± 8**
100	500	0	51 ± 5*	61 ± 5**
200	250	0	52 ± 2*	59 ± 10**
0	250	3000	43 ± 6*	69 ± 2**
0	500	6000	61 ± 3*	62 ± 5**
0	500	3000	49 ± 2*	71 ± 5**
0	250	6000	52 ± 5*	60 ± 6**
100	250	3000	53 ± 1*	55 ± 3**
200	250	3000	47 ± 1*	57 ± 15**
100	250	6000	49 ± 1*	61 ± 3**
200	250	6000	53 ± 3*	63 ± 6**
100	500	3000	63 ± 4*	65 ± 10**
200	500	3000	49 ± 5*	66 ± 2**
100	500	6000	55 ± 6*	66 ± 1**
200	500	6000	54 ± 4*	61 ± 7**

[a] ^{14}C uptake is presented as % control without complexing agents; 100% = 55 894 dpm.
[b] ^{14}C uptake is presented as % control with elutriate; 100% = 49 063 dpm.
[c] Control.

predicted response, a_x = observed response for the individual toxicant x, and b_y = observed response for the individual toxicant y.

In general, most Cu, Zn, and Pb interactions were antagonistic according to all three methods. However, Zn (250 $\mu g \cdot L^{-1}$) when combined with either Pb (3000 $\mu g \cdot L^{-1}$) or Pb (6000 $\mu g \cdot L^{-1}$) behaved additively. Competition for complexing ligands may result in either antagonism or synergism between metals. The majority of metal interactions in the presence of algal exudates were also antagonistic. Competition between Cu, Zn, and Pb for the same functional groups in the algal exudates or at the cell surface caused a net decrease in free ion concentrations of all three metals, which resulted in a decrease in their joint toxicity, i.e. an antagonistic response. Conversely, a synergistic response can arise from the same competition between metals for binding sites. However, in this case, one metal successfully outcompetes another, resulting in an increase in free metal concentration and in its toxicity. Synergism between mixtures of Zn and Pb in the presence of algal exudates was determined by the Colby (1967) method. Synergistic responses were also calculated with the method of Voyer and Heltshe (1984) for combinations of Cu (100 $\mu g \cdot L^{-1}$) plus Zn (250 $\mu g \cdot L^{-1}$) plus Pb (6000 $\mu g \cdot L^{-1}$) and Cu (100 $\mu g \cdot L^{-1}$) plus Zn (500 $\mu g \cdot L^{-1}$) plus Pb (6000 $\mu g \cdot L^{-1}$) with complexing agents. This observed synergism may be related to the preferential binding of Zn and Cu by algal exudates rather than Pb when exposed to all three metals simultaneously (Table 2).

Metal interactions in the presence or absence of sediment elutriate were identical. All interactions appeared to be antagonistic, except for those of Cu (100 $\mu g \cdot L^{-1}$) plus Pb (3000 $\mu g \cdot L^{-1}$), Cu (100 $\mu g \cdot L^{-1}$) plus Zn (500 $\mu g \cdot L^{-1}$), and Cu (100 $\mu g \cdot L^{-1}$) plus Zn (500 $\mu g \cdot L^{-1}$) plus Pb (3000 $\mu g \cdot L^{-1}$) which were additive. The relative concentrations of individual metals in a mixture determined the metal–metal interaction.

TABLE 6. Heavy metal toxicity to growth of *S. quadricauda* at pH 4.5, 6.5, and 8.5. *Significantly different from control pH 4.5 at 95% C.I. (Student *t*-test); **significantly different from control pH 6.5 at 95% C.I. (Student *t*-test); ***significantly different from control pH 8.5 at 95% C.I. (Student *t*-test).

Metal ($\mu g \cdot L^{-1}$)			Growth rate (SD)[a] ($N = 3$)		
Cu	Zn	Pb	pH 4.5	pH 6.5	pH 8.5
0	0	0	0.194 (0.027)	0.171 (0.001)	0.154 (0.017)
50	0	0	0.167 (0.010)	0.181 (0.033)	0.144 (0.013)
100	0	0	0.018 (0.018)*	0.244 (0.001)**	0.168 (0.021)
200	0	0	0.000 (0.000)*	0.047 (0.007)**	0.197 (0.026)
0	100	0	0.100 (0.011)*	0.101 (0.030)	0.122 (0.013)
0	225	0	0.040 (0.009)*	0.042 (0.011)**	0.131 (0.029)
0	500	0	0.000 (0.000)*	0.026 (0.013)**	0.109 (0.004)***
0	0	1000	0.173 (0.004)	0.140 (0.010)**	0.147 (0.001)
0	0	3000	0.141 (0.019)	0.148 (0.000)**	0.077 (0.010)***
0	0	6000	0.028 (0.013)*	0.135 (0.002)**	0.063 (0.007)***
100	100	0	0.009 (0.009)*	0.086 (0.032)**	0.137 (0.003)
100	225	0	0.004 (0.004)*	0.061 (0.010)**	0.107 (0.011)***
100	500	0	0.000 (0.000)*	0.000 (0.000)**	0.127 (0.050)
100	0	1000	0.011 (0.011)*	0.244 (0.005)**	0.183 (0.001)
100	0	3000	0.016 (0.016)*	0.196 (0.020)	0.088 (0.017)***
100	0	6000	0.000 (0.000)*	0.280 (0.012)**	0.093 (0.017)***
50	225	0	0.014 (0.000)*	0.035 (0.032)**	0.105 (0.007)
200	225	0	0.000 (0.000)*	0.114 (0.006)**	0.136 (0.007)
0	225	1000	0.029 (0.003)*	0.043 (0.004)**	0.110 (0.013)***
0	225	3000	0.007 (0.007)*	0.013 (0.005)**	0.134 (0.004)
0	225	6000	0.000 (0.000)*	0.069 (0.017)**	0.070 (0.004)***
50	0	3000	0.156 (0.033)*	0.288 (0.001)**	0.069 (0.004)***
200	0	3000	0.035 (0.008)*	0.113 (0.011)**	0.154 (0.033)
0	100	3000	0.038 (0.012)*	0.058 (0.016)**	0.129 (0.022)
0	500	3000	0.016 (0.016)*	0.012 (0.006)**	0.039 (0.017)***
100	225	1000	0.016 (0.016)*	0.049 (0.003)**	0.119 (0.013)
100	225	3000	0.000 (0.000)*	0.083 (0.006)**	0.150 (0.041)
100	225	6000	0.000 (0.000)*	0.086 (0.030)**	0.136 (0.017)
100	100	3000	0.011 (0.011)*	0.090 (0.006)**	0.120 (0.001)***
100	500	3000	0.006 (0.006)*	0.027 (0.001)**	0.125 (0.014)
50	225	3000	0.011 (0.011)*	0.075 (0.001)**	0.109 (0.006)***
200	225	3000	0.000 (0.000)*	0.038 (0.024)**	0.111 (0.035)***

[a] $\mu_{avg} = (\ln X(T) - \ln X_0)/T$ (Nyholm 1985).

pH

The influence of pH on long-term metal toxicity to growth of *S. quadricauda* was also investigated. In general, the toxicity of individual metals and metal mixtures increased with a corresponding increase in acidity (Table 6). For example, even though the highest growth rate for control cells was observed at pH 4.5, no growth was observed for algal cells exposed to either Cu (200 $\mu g \cdot L^{-1}$) or Zn (500 $\mu g \cdot L^{-1}$) at the same pH. The final pH (after 15 d) for all control tubes regardless of initial pH was about 8.0–8.5. The final pH of tubes with an initial pH of 8.5, 6.5, and 4.5 was approximately 8.0, 7.5–8.0, and 6.0, respectively.

Acidification can influence metal toxicity by either affecting metal speciation or the biological sensitivity at the cell surface (Campbell and Stokes 1985). Increasing acidity increases the free metal ion concentration in solution, due to competition between bound metal ions and free H^+ for negatively charged exchange sites on organics and inorganics. Under acidic conditions, metals tend to exist in the free ionic form which is believed to impart metal toxicity. However, reports on the effect of pH on metal toxicity have been inconclusive (Steemann Nielsen and Kamp-Nielsen 1970; Gachter 1976; Hargreaves and Whitton 1976a, 1976b; Monahan 1976; Harding and Whitton 1977; Rai et al. 1981). Peterson et al. (1984) suggested that competition between heavy metals and H^+ for the same cellular binding sites resulted in a decrease in cellular heavy metal uptake and toxicity. Recently, Campbell and Stokes (1985) noted the effect of acidification on the speciation of several metals. With a drop in pH from 7 to 4, significant speciation changes were observed for both Cu and Pb. At pH 7, approximately 37% of Cu and Pb in solution was present as the free aquo ion, while at pH 4 and 5 the concentration of free ionic species increased to 100%. Conversely the speciation of Zn was not affected by acidification and was present 100% as the free aquo ion at pH 4–7.

If the decrease in pH did not alter metal (Zn) speciation, then the observed increase in toxicity at pH 4.5 resulted from a heightened sensitivity of the algal cells to the metal. Altered membrane potentials at acidic pH may affect metal uptake (i.e.

TABLE 7. Effect of pH on heavy metal interactions towards algal growth (long-term exposure) determined by three methods. A = antagonistic; S = synergistic; + = additive.

Metal ($\mu g \cdot L^{-1}$)			Colby (1967)			Borgmann (1980)			Voyer and Heltshe (1984)		
Cu	Zn	Pb	pH 8.5	pH 6.5	pH 4.5	pH 8.5	pH 6.5	pH 4.5	pH 8.5	pH 6.5	pH 4.5
100	100		+	+	+	A	+	+	A	+	+
100	225		S	+	+	S	+	S	S	+	+
100	500		+	S	+	S	S	S	+	S	+
100		1000	+	A	+	+	A	+	A	A	+
100		3000	+	+	+	S	+	+	+	+	+
100		6000	A	A	S	+	A	S	+	A	+
50	225		S	+	S	S	+	S	S	+	+
200	225		S	A	+	S	A	S	+	A	+
	225	1000	+	+	+	+	+	S	+	A	A
	225	3000	A	S	S	A	S	S	A	+	+
	225	6000	A	+	S	A	+	S	A	A	+
50		3000	+	A	+	+	A	+	+	A	S
200		3000	+	A	A	A	A	S	A	A	A
	100	3000	+	+	S	+	S	S	A	+	+
	500	3000	+	+	+	+	+	S	+	+	+
100	225	1000	A	+	+	S	+	+	+	A	+
100	225	3000	A	A	S	+	A	S	A	A	+
100	225	6000	A	+	+	A	+	S	A	+	+
100	100	3000	A	S	+	A	S	+	A	+	+
100	500	3000	A	S	+	A	+	+	A	A	+
50	225	3000	A	A	+	A	A	S	A	A	+
200	225	3000	+	+	+	+	+	S	+	A	+

increase metal uptake) and may explain the increase in metal toxicity. Changes in pH may also cause conformational changes in the metal binding sites at the cell surface, thereby altering algal cell binding capacities for metals. The observed increase in individual Cu and Pb toxicity at pH 4.5 is believed to result from the dissociation of metal complexes formed at higher pH. Thus, the bioavailability of the toxic free metal ion is enhanced.

All toxic effects exerted by combinations of Cu, Zn, and Pb to growth were enhanced at pH 4.5. Due to the length of the exposure period (15 d) to assess the effect of pH on metal toxicity, algal metabolic products capable of complexing metals accumulated in the growth medium. An increase in complex stability has been observed at high pH (Cheng et al. 1975). The formation of stable metal–organic complexes by these algal metabolic products at alkaline pH contributed to the reduced metal toxicity. Under acidic conditions, competition may occur not only between H^+ and metal ions for active sites on the cell surface, but also for weakly acidic ligands (Gould and Genetelli 1978). Competition between the added metals and H^+ for complexing ligands present in the accumulated algal metabolic products may influence metal toxicity to algal growth.

Metal interactions at pH 8.5, 6.5, and 4.5 were compared (Table 7). In general, at pH 8.5 and 6.5 for all methods employed, Cu, Zn, and Pb behaved both antagonistically and additively. Few synergistic interactions occurred, primarily between Cu and Zn. At pH 4.5, however, the metal interactions changed so that synergism predominated, especially for those interactions determined according to the method of Borgmann (1980). Interactions determined by the method of Voyer and Heltshe (1984) suggest that Cu, Zn, and Pb behave additively at acidic pH. Determination of metal interactions was dependent on the method chosen to analyse the data. Changes in pH did alter the interactions between Cu, Zn, and Pb to algal growth. Enhanced toxicity of individual and multiple metals was observed at pH 4.5.

Continuous loading of heavy metals and other toxicants into the aquatic environment has created an urgent need for future research to focus on the interactive effects of these pollutants on the aquatic biota. The data presented emphasize that the physico-chemical properties of an aquatic ecosystem determine the speciation and hence toxicity of metals in the aquatic environment. Competition between metals can result in either synergistic, antagonistic, or additive interactions, depending on the concentration and speciation of the respective metals.

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Factors Affecting the Distribution, Abundance, and Measurement of Pacific Herring (*Clupea harengus pallasi*) Spawn

D. E. Hay and A. R. Kronlund[1]

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

Hay, D. E., and A. R. Kronlund. 1987. Factors affecting the distribution, abundance, and measurement of Pacific herring (*Clupea harengus pallasi*) spawn. Can. J. Fish. Aquat. Sci. 44: 1181–1194.

Records of the date, location, and magnitude of Pacific herring (*Clupea harengus pallasi*) spawnings in British Columbia, collected since 1928, were compiled and analysed. In the early years of spawn surveys, adjacent spawnings were often reported as single events. Gradually, this practice has changed so that each spawning has a separate record. As a consequence of this change in methods, the mean length of spawnings has decreased in recent years but the total numbers of records has increased. Estimates of mean spawning width and intensity have also changed, partly due to changing survey methods as well as changing spawning distributions. A spawn abundance index is developed to account for these temporal changes. Abiotic factors affecting the distribution and abundance of spawn deposition include sea surface temperatures and the fishery. Biological factors affecting spawn distribution and abundance are not as well defined, but it is shown that in some situations, spawn dimensions may change as a function of stock abundance.

Les auteurs ont compilé et analysé les données enregistrées depuis 1928 sur la date, le lieu et l'importance du frai du hareng de Pacifique (*Clupea harengus pallasi*) en Colombie-Britannique. Il est souvent arrivé, au cours des premières années où l'on a effectué ces inventaires, que des frais se déroulant dans des zones très voisines soient signalés comme des événements uniques. Cette pratique s'est graduellement modifiée de sorte que chaque ponte a été signalée individuellement. Il en est résulté au cours des dernières années une diminution de la longueur moyenne des pontes et une augmentation du nombre total d'événements signalés. Les estimations de la largeur et de l'intensité moyennes des pontes ont aussi été modifiées, en partie à cause du changement de méthodes d'inventaire et de la répartition des pontes. Les auteurs ont établi un indice d'abondance des pontes pour tenir compte de ces variations temporelles. La température de l'eau de surface et l'importance des pêches sont des facteurs abiotiques influant sur la répartition et l'abondance des pontes. Les facteurs biologiques ne sont pas aussi bien définis, mais les auteurs montrent que, dans certaines situations, la dimension des pontes peut varier en fonction de l'abondance du stock.

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This paper examines abiotic and biotic factors affecting the distribution and abundance of spawn of the Pacific herring (*Clupea harengus pallasi*) and examines factors affecting the measurement of spawn deposition. An index of spawn deposition is developed which allows a temporal comparison of relative spawn deposition, among small geographical units, since the spawn surveys were started in the late 1920s and early 1930s.

Pacific herring spawn during spring months in intertidal and subtidal locations. The spawns are usually conspicuous: milt makes the water milky white and often large concentrations of seabirds are attracted to the spawning site. Records of the locations, data, and magnitude of the spawns began in the late 1920s. The records were collected by Fishery Officers working and residing throughout the British Columbia coast. The widespread distribution of Fishery Officers reflects the geographically diverse fisheries for Pacific salmon (*Oncorhynchus* spp.) and associated rivers and streams. The distribution of Fishery Officers was also ideal for monitoring the annual herring spawning activity throughout many remote coastal locations.

The original intention of collecting the spawning records was as an informal guide to the spawning escapement from the fisheries in the previous fall and winter. Since spawn assessments began, different quantitive methods were used to assess spawn. Each method was based on different assumptions about the spawning biology. This paper provides additional information about herring spawning biology, much of it based on spawning records. These data are supplemented with field tests and observations. An index of spawn deposition is used to examine temporal changes in relative spawn deposition within and among different geographical areas. Abiotic and biotic factors that could affect spawn deposition are examined and discussed.

Methods

In 1982 we began a review of all British Columbia herring spawning records. At that time, the retrievable data base began

[1] Present address: Department of Mathematics and Statistics, University of Guelph, Guelph, Ont. N1G 2W1.

TABLE 1. Herring spawn intensity classifications showing the pre- and post-1969 scales, empirical estimates of eggs, and a relative weighting scale.

Pre-1969 scale	Post-1969 scale	Description	Number of eggs/m^2	Weighting factors
1	1	Very light	50 100	0.05
	2	Very light to light	182 600	0.20
2	3	Light	421 300	0.40
	4	Light to medium	665 200	0.65
3	5	Medium	1 008 500	1.00
	6	Medium to heavy	1 417 000	1.40
4	7	Heavy	1 889 000	1.90
	8	Heavy to very heavy	2 423 200	2.40
5	9	Very heavy	3 018 400	3.00

in the year 1951 although Fishery Officer records were available for many years between 1928 and 1950. These earlier records were added to the data base and all measurements and dimensions of spawns were converted to metric units. From 1928 until 1984 a total of 18 822 records of spawnings were made from 1114 locations throughout the British Columbia coast. Each complete record consists of the following information.

(1) The year, month, and day of the initiation of spawning.

(2) The location of spawnings, according to (i) statistical area, (ii) section (Fig. 1), and (iii) specific geographical location which is coded with a unique number (Hourston and Hamer 1979).

(3) The length of spawns. Most Fishery Officer records made during the early years were accompanied by charts or facsimilies which showed the exact locations of spawns. When the charts were available the spawn lengths indicated on the charts were compared with the quantitative estimate made in yards or miles. Usually the two estimates of spawn length were identical or close. In a few instances, discrepancies occurred and the estimate used in our analysis was based on the length determined from the charts. Most of the suspected errors were incorrect conversions from the unit of original length estimation (feet, yards, statute and nautical miles) to the unit reported.

(4) Spawn width. Width estimates were not made during the earliest years of records. At that time it was believed that length estimates were just as accurate as area estimates (Stevenson and Outram 1953; Tester 1948). However, a space for an estimate of width was on the forms provided to Fishery Officers in the late 1930s, and the Officers were requested to make estimates of average width. Most records since 1940 include width estimates.

(5) Intensity. From 1928 until 1969, intensity was assessed on a scale of 1–5. The instructions on the 1951 Fishery Officer survey form read as follows: "Estimate the intensity of spawn deposition in the usual five categories — very light, light, medium, heavy and very heavy." A few eggs per blade of eelgrass or rockweed (1–25) would be classified as light. A coating of several layers of eggs was classified as very heavy. Very light to heavy was classified as 1–5, respectively. In 1969, the forms changed to allow a total of nine intensity categories (Table 1). There was no documentation about the rationale for this change, but it was probably to accommodate the practice of reporting intermediate categories (such as 3.5 for an intensity between 3 (medium) and 4 (heavy). In 1981 the intensity classification was abandoned altogether and replaced with an estimate of "egg layers." Such egg layer estimates are useful for obtaining estimates of total egg counts per unit area (Haegele et al. 1979). As a consequence, very few records made subsequent to 1981 have intensity estimates. To compare the pre- and post-1969 intensity scales, all pre-1969 intensities were converted to the post-1969 scale (1–9) (Table 1). For our analyses, we used a weighted estimate of intensities based on earlier work in which empirical counts of egg densities (eggs per square metre) were made for each of the nine intensity classifications (Hourston et al. 1972; Humphreys and Haegele 1976). For instance, mean egg densities were about 3 million at "very heavy," 1 million at "medium," and 50 000 at "very light." However, absolute egg densities could vary according to a host of factors including the type of substrate. Therefore, we used a relative weighting (adapted from Chalmers and Sprout 1981) in which the mean egg densities at each classification were divided by 10^6. For instance, "medium" intensity is set at 1.0, "very heavy" at 3.0, "very light" at 0.05, etc. (Table 1).

Review and Analyses of Previous Spawn Indices

Estimates of spawn escapement have been published annually: from 1937 until 1957 in series published by the British Columbia Fisheries Department (i.e. Taylor et al. 1957) and from 1955 to the present in various informal series published by the Federal Department of Fisheries and Oceans (i.e. Anonymous 1984). Tester (1948) was the first to comment on a series of years of spawning data (1931–46) for the west coast of Vancouver Island. He proposed two indices of spawn deposition: (1) the cumulative length of spawn and (2) the cumulative length adjusted for variation in spawn intensity. His main conclusion was that spawning escapement varied substantially among years and that the magnitude of escapement had little relationship with subsequent year-class success.

Stevenson and Outram (1953) developed a relationship between the length of spawn and the number of fish that spawn in a statute mile. They adjusted for differences in intensity: the lengths of spawns with heavy intensities were weighted more heavily than spawns with light intensity. The adjustment involved an increase or decrease in the observed length as a function of intensity; the result was an estimated spawn length in statute miles, adjusted to "medium" intensity. Taylor (1964) extended the analysis using 26 yr of spawning data (1937–62) for seven different subdivisions of the British Columbia coast. He used the same measure of spawn (statute miles adjusted for intensity). Hourston et al. (1972) reanalyzed the spawn data and incorporated data on spawn widths: for each spawn record an estimate of total spawn area was made and the area was

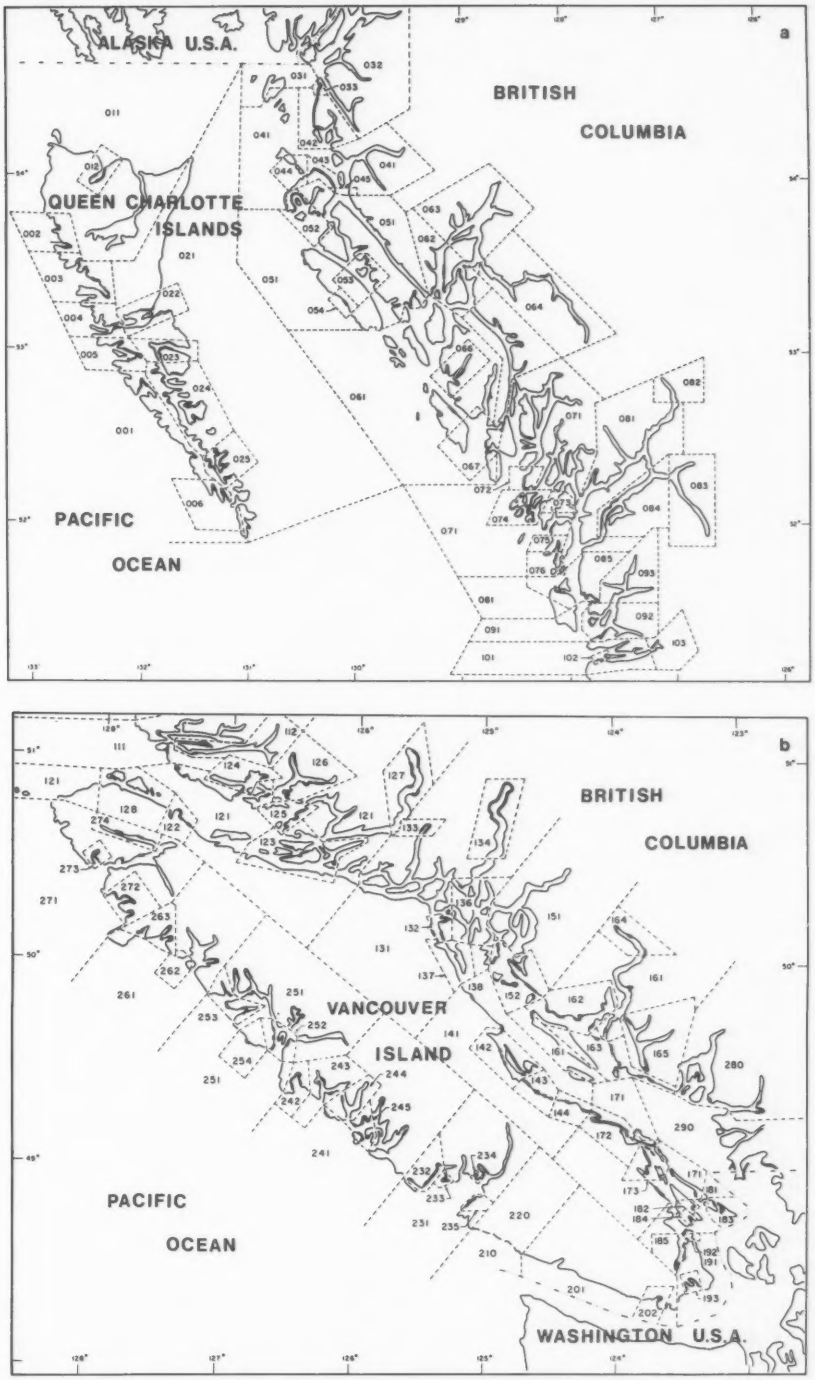

FIG. 1. Statistical areas and sections of (a) northern and (b) southern British Columbia. The first two numbers of each division represent the statistical area (from 0 in the north to 29 in the south). The last number indicates a subdivision of the statistical area into two or more sections. Areas 28 (280) and 29 (290) are the only areas not subdivided into sections. The darkened (thickened) edges indicate spawning areas.

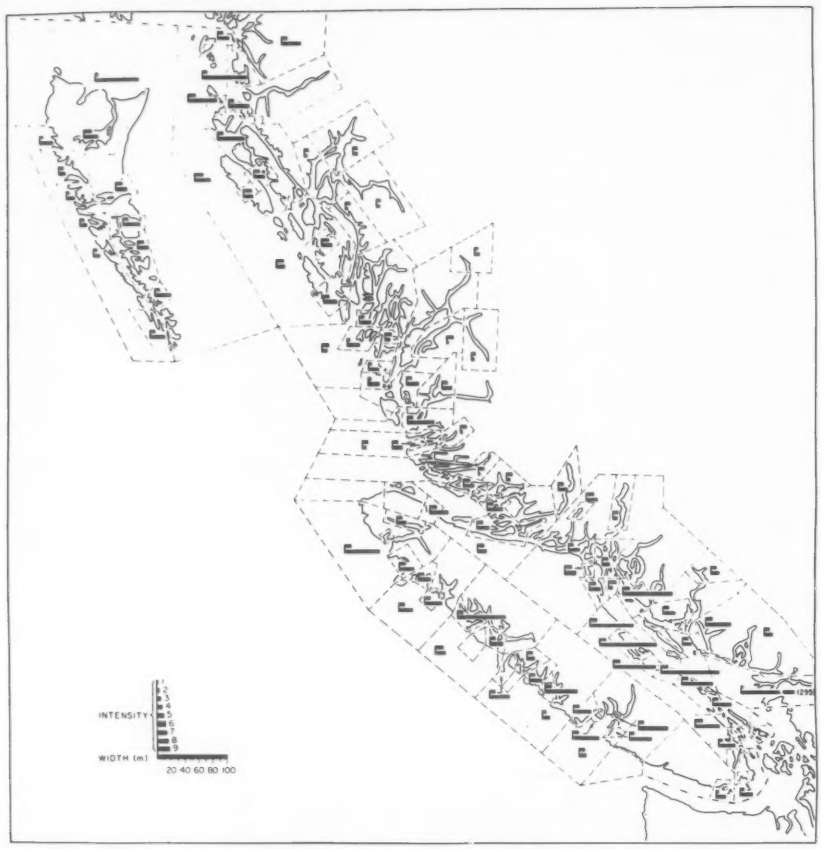

FIG. 2. Geographic variation in width and intensity among sections. The upper horizontal bar represents the mean spawn intensity; the lower bar represents mean width.

weighted according to the spawn intensity. Further, the British Columbia coast was divided into 110 different sections that were based mainly on the centers of spawning (Hourston and Hamer 1979). These "spawning sections" were described as geographical subdivisions of the well-established "statistical areas" (Fig. 1). This approach was developed and applied in the early 1970s and was the most comprehensive and biologically realistic of any developed. Spawn data from 1951 were used and the data were grouped according to meaningful geographical divisions called sections. Estimates of spawn deposition were then incorporated into a model which also considered age and size data and size-specific fecundity data to estimate the biomass of spawning escapement and forecast future runs (Hourston 1981; Hourston and Schweigert 1981). Recently this approach has been modified and extended by Schweigert and Stocker (1987), who developed a method of adjusting width and intensity estimates according to empirical observations and comparisons of Fishery Officer and SCUBA diver surveys. The intent of their work is an estimate of the absolute tonnage of the herring spawning biomass.

Temporal and Geographical Variation in Spawn Records

There are significant differences in the estimates of a average width and intensities among different sections of the coast (Fig. 2). In general, widths and intensities are greatest in the Strait of Georgia (especially Statistical Area 14) where much of the spawning occurs on wide, shallow intertidal and subtidal areas. Widths and intensities are often lower in inlets or fjords which have steep sides and narrow vegetative zonation; however, average spawn lengths in these locations are often the greatest. Therefore, the dimensions of spawns are partially dependent on the bottom topography as well as the distribution and type of vegetation present.

The spawn data also reflect changes in the survey methods. One consequence is a gradual reduction in mean spawn length over time (Fig. 3a). During the earlier years of the surveys (pre-1960s), Fishery Officers were instructed to pool the lengths of adjacent spawns that occurred in the same general vicinity at the same time. In recent years, Fishery Officers have been encouraged to make their observations and reports as thorough as possible. As a consequence, the more recent data consist of a larger number of smaller spawns. The *cumulative* length of spawn (Fig. 3b) is a better comparison of spawn deposition between years. However, some remote areas may have incomplete records, especially the west coast of the Queen Charlotte Islands, where there were relatively few records prior to 1970. Therefore, estimates of total spawn lengths for the whole coast are incomplete, particularly for

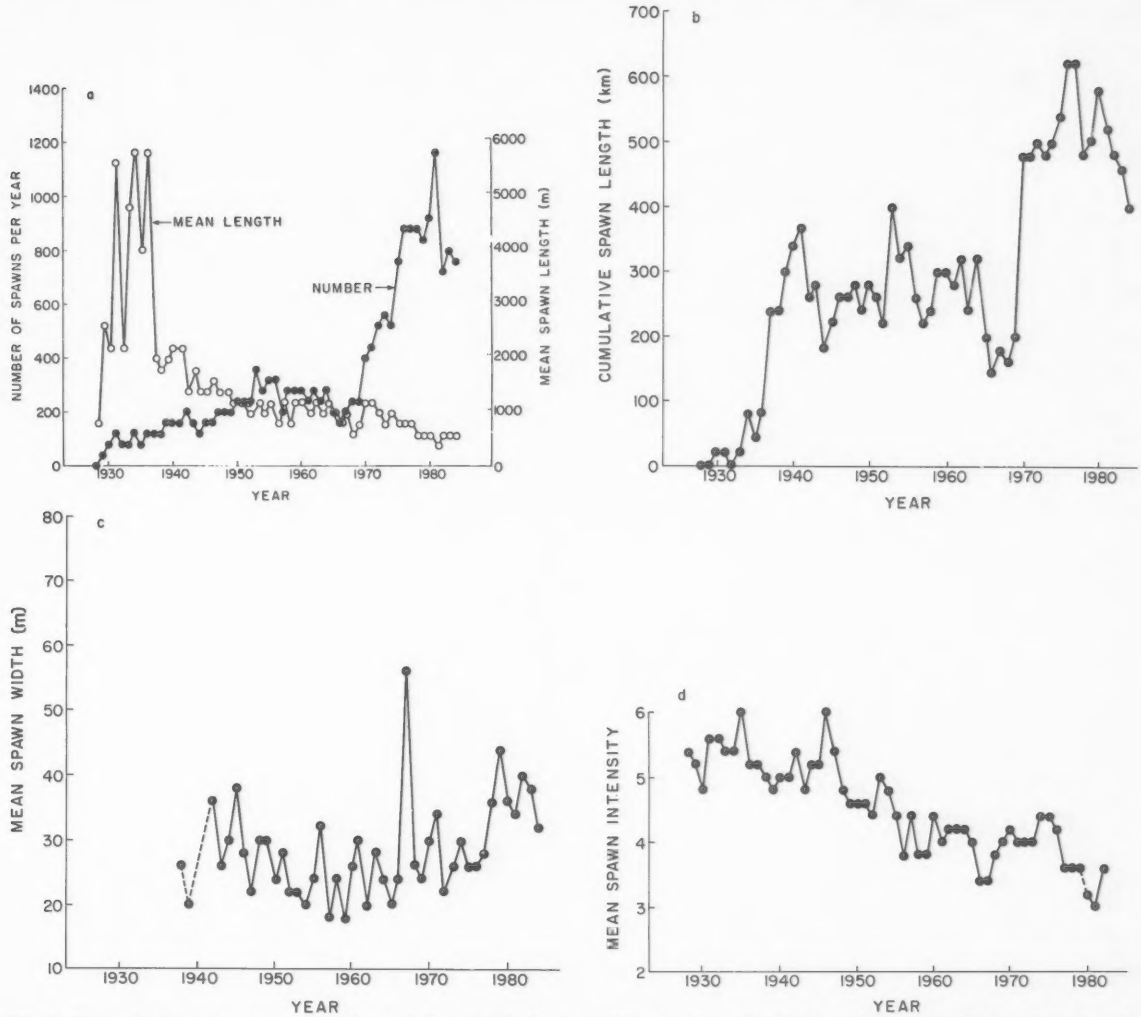

FIG. 3. Temporal changes in spawn parameters. (a) Mean length of spawns (○) and total number of spawns (●) by year; (b) cumulative length of spawn by year; (c) mean widths of spawn by years (very few records made before 1940 (broken line) included width estimates); (d) mean spawn intensity by year.

earlier years. Total length estimates for some specific regions, such as the Strait of Georgia, probably are more complete. The cumulative annual spawn length, however, can be misleading as a spawn index because spawn locations vary in width and intensity. There are also temporal differences in estimates of mean spawn width and intensity: width estimates increase slightly with time from 1960 to 1980 (Fig. 3c) and intensity estimates decrease with time (Fig. 3d). The explanation for temporal variation in width and intensity could involve several different factors: (1) changes in methods of surveys; (ii) shifts in spawn deposition among locations; and (iii) real differences in the spawn deposition patterns perhaps as a consequence of changes in stock abundance. Each of the possibilities is vital to the development of an index of spawn deposition and each is discussed below.

At least three changes have occurred in spawn surveys that could affect width and intensity estimates. First, the greater number of spawns reported for recent years reflects a methodological change towards recording shorter spawns as separate events rather than pooling, as was the practice in earlier years. Therefore, the greater number of shorter spawns in recent years also would have included a greater number of records of spawns with lower intensities. Second, the change of intensity scales from a five-scale system to a nine-scale system in 1969 could have promoted conservatism by the Fishery Officers. When faced with a choice of nine possible intensity ratings with five as the median, there may have been a reluctance to utilize the extremes of the newly introduced system. There is a higher frequency of extremes (1s and 5s) in the five-scale system than the extreme (1s and 9s) in the nine-scale system. The third possible methodological change involves the gradual recognition of the magnitude of the subtidal component of spawns. Haegele et al. (1981) showed that spawn intensity was greatest at chart datum (approximately at

TABLE 2. Changes in width and intensity over time within spawning locations. Of a total of 1114 locations recorded since 1928, the most important (i.e. those having more than 70 records) are shown here. Most locations had more than one spawn record per year. Information for each location includes (i) the number of records, (ii) the number of years over which the records were made, (iii) the mean, (iv) standard error (SE), (v) regression coefficient or slope of width by year and intensity by year, and (vi) the correlation coefficient (r^2) multiplied by 100.

		Width						Intensity					
Section	Location name	No. of records	Years	Mean	SE	Slope	r^2 (×100)	No. of records	Years	Mean	SE	Slope	r^2 (×100)
002	Port Louis	193	20	21.9	4.1	+0.06	0.0	74	17	2.0	0.2	+0.07	16.9
005	Inskip Ch.	95	18	11.8	1.2	+0.44	1.2	74	15	4.0	0.2	−0.10	10.2
006	Louscoone In.	170	26	19.6	1.8	+0.66	3.0	146	23	3.8	0.1	−0.11	17.8
033	Stuman Bay	102	24	31.1	6.6	−0.03	0.0	91	22	3.8	0.4	−0.04	5.3
042	Big Bay	99	41	72.5	10.3	+1.22	2.8	84	38	4.4	0.5	−0.12	63.6
052	Kitkatla In.	83	22	24.7	2.1	−0.16	0.6	84	25	4.6	0.5	+0.02	0.8
052	Gurd Is.	96	23	32.8	3.9	+0.98	6.7	95	22	5.0	0.2	−0.01	0.8
067	Wilby Pt.	70	20	17.6	3.8	−0.41	1.8	54	22	3.8	0.3	−0.11	61.0
067	Parsons Anch.	95	26	20.3	2.3	−0.04	0.0	73	23	3.4	0.2	−0.13	54.1
067	Weeteem Bay	110	9	18.8	2.4	+2.93	5.7	81	6	2.9	0.2	−0.41	17.4
067	Higgins Pass	134	28	17.8	1.3	−0.04	0.1	119	25	4.9	0.2	−0.07	23.4
074	Cape Mark	76	9	32.5	4.8	−0.57	0.2	61	8	3.5	0.1	+0.13	0.1
074	Thompson Bay	76	20	11.2	2.8	+0.68	7.4	73	19	3.9	0.2	−0.06	10.4
075	McNaughton Gp.	84	19	15.2	2.5	+0.18	0.4	74	16	3.9	0.2	−0.08	10.7
076	Hurricane Is.	75	18	19.4	4.0	+0.18	0.1	61	16	3.5	0.2	−0.11	22.5
083	N. Bentinck Arm	101	27	4.0	0.3	−0.01	0.2	97	28	2.4	0.1	−0.05	17.4
084	Burke Ch.	135	17	3.5	0.2	−0.09	2.9	119	18	5.3	0.1	+0.03	1.6
085	Illahie In.	85	22	7.6	1.2	−0.14	1.5	72	21	4.1	0.2	−0.08	16.9
085	Kwakume Pt.	87	10	17.8	3.4	−0.14	0.0	77	8	3.9	0.2	−0.17	8.4
093	Rivers Inlet	96	35	18.1	3.6	+0.26	0.6	95	40	3.6	0.2	−0.03	3.5
102	Takush Hbr.	70	25	7.3	1.3	+0.07	0.4	72	30	4.4	0.2	−0.01	1.2
122	Fort Rupert	76	26	25.7	4.3	−0.46	1.9	71	25	4.1	0.2	−0.03	5.9
126	Wakeman Sd.	121	41	4.3	0.5	−0.46	0.2	90	39	4.3	0.2	−0.08	37.7
127	Knight Inlet	170	33	14.0	3.6	+0.29	0.4	165	37	4.0	0.1	−0.06	16.5
137	Hyacinthe Bay	79	25	15.5	2.4	+0.59	7.7	82	35	4.6	0.2	−0.05	12.0
142	Comox Hbr.	63	23	130.4	21.6	+7.42	19.2	71	34	3.8	0.2	−0.04	6.2
152	Scuttle Bay	63	30	113.8	16.5	+1.84	3.5	70	38	5.1	0.2	−0.05	8.4
165	Porpoise Bay	67	37	38.2	5.8	−0.88	3.4	84	46	4.5	0.2	−0.06	17.1
172	Nanoose Bay Head	79	27	57.2	8.8	+3.50	14.8	77	27	3.9	0.2	−0.005	6.1
173	Ladysmith Hbr.	79	27	27.3	6.1	−1.77	9.6	96	35	4.5	0.2	−0.06	9.3
173	Kulleet Bay	80	30	26.2	3.4	+0.50	2.8	81	32	4.3	0.2	−0.06	9.0
173	Coffin Pt.	86	35	23.4	4.1	+1.40	20.2	79	37	4.4	0.3	−0.10	25.1
182	Long Hbr.	136	27	17.7	2.0	−0.41	2.0	139	29	3.3	0.1	+0.01	0.3
182	Annette Inlet	149	37	21.8	2.3	−0.36	1.8	145	35	2.5	0.1	+0.00	0.0
232	Stopper Is.	75	18	21.2	4.0	+0.64	3.0	67	19	3.9	0.2	−0.03	3.7
232	Maggie River	92	30	24.7	3.8	+0.98	12.0	88	36	3.8	0.2	−0.02	2.2
232	Toquart Bay	118	35	19.7	2.9	−1.7	2.0	126	44	4.4	−0.2	−0.02	1.5
242	Hesquiat Hbr.	88	25	29.2	3.4	+0.44	1.6	95	31	4.0	0.4	−0.01	0.8
243	Hot Springs Cove	91	21	11.2	1.7	−0.45	13.2	65	26	4.3	0.2	−0.03	8.9
245	Vargas Is.	105	12	68.3	6.5	+0.72	0.1	91	12	5.0	0.2	+0.00	0.0
253	Nuchatlitz In.	77	13	107.5	15.3	+1.95	2.8	64	16	4.2	0.2	−0.04	7.4
253	Outer Nuchatlitz	86	22	95.7	8.8	+4.99	27.6	63	19	3.3	0.3	−0.14	13.3
253	Nuchatlitz Village	99	27	84.8	10.7	+0.96	0.9	84	26	4.7	0.2	−0.10	20.2
253	Port Langford	122	34	50.4	5.2	+1.51	8.9	84	32	3.8	0.2	−0.02	1.8

the edge of the water at low tide). Spawn intensity gradually decreases in higher intertidal and lower subtidal locations. Prior to 1970, spawn surveys were conducted mainly from the shore, especially at low tide. Observations would have been made mainly on the higher intensity spawn above chart datum. Since 1970, spawn surveys generally made greater efforts to estimate spawn intensity and rakes were used to sample subtidal vegetation. It is possible that the greater attention paid to the lower intensity subtidal spawn in recent years could have resulted in a gradual decreasing estimate of intensity. This argument applies also to estimates of spawn width: the tendency in early years (1940s–1960s) to underestimate the subtidal spawn component could have resulted in some underestimates of spawn width.

Geographical Variation in Spawn Deposition

Annual changes in the relative quantity of spawn deposition among locations could give the appearance of changes in the spawn lengths, widths, or intensities. For instance, spawn in certain inlets is limited to narrow widths and light intensities because of topographical limitations in the distribution and abundance of vegetation as spawn substrate. Changes in local stock abundance leading to increased or decreased spawning in such inlets would then have the effect of changing the mean

annual spawn widths or intensities when specific locations are included in the analysis of larger areas. For example, the extremely high width estimates in 1967 (Fig. 3c) were a consequence of unusual spawning on the extensive tidal flats of Boundary Bay (Area 29). Eight records made there in 1967 are unique in the data series with extraordinarily high widths (>1 km) and very light intensities. Gradual changes or cycles in the geographical spawning locations could partially explain the long-term trend for the decrease in mean spawn intensity and the increase in mean spawn width. For instance, since 1970 in the Strait of Georgia, there has been a substantial reduction in spawning in Statistical Area 18, which is characterized by narrow spawns of light intensity. At the same time, there has been an increase in spawning in Area 14, characterized by wide spawns of higher intensity. Therefore, data pooled from these areas will give the appearance of a change in the spawn deposition parameters whereas the real change was in the relative sizes of stocks between areas.

A total of 1114 individual locations were identified as having received one or more spawnings since 1928. To estimate the consistency of the spawn deposition patterns for single locations, we examined the annual trend in spawn width and intensity for each location. Representative locations with 70 or more spawning records are listed in Table 2. In most locations there is a weak or negligible change in width over time. The temporal trend in intensity is slight, but most locations show a negative slope for the regression line of intensity over time. For individual locations the trends for changes in spawn deposition parameters are more conservative than for large regions. Therefore, changes over time in the estimates of spawn widths and intensity are a consequence of shifts of spawn among locations as well as the recent trend to report more detail on individual spawns — resulting in a greater number of observations of smaller spawns.

An Index of Spawn Abundance

For any subdivision of the coast a spawn coefficient (SC) can be defined as the mean of the product of spawn width (W) and the weighted intensity (I) for all spawn records that have both width and intensity estimates:

$$SC = \frac{1}{n} \sum_{j=1}^{n} (W_j I_j)$$

where n is the number of records used. An index of total spawn (SI) deposition in year k is SI_k. For year k it is defined as the cumulative length of spawn (L_k) within a unit area multiplied by the area-specific spawn coefficient:

$$SI_k = L_k \cdot SC.$$

This index of spawn deposition then can be calculated for any year on any part or parts of the coast.

There remains, however, the problem of deciding the optimal number and size of geographical units over which to apply the index. That is, the area specific constants could be calculated at the level of the 1114 individual locations, or approximately 110 sections (Fig. 1). From Table 2 it is clear that width and intensity vary within locations, but generally, variation among locations exceeds variation within. To assist with this decision the spawn coefficient was calculated and integrated first at the location level (1114 locations) and then

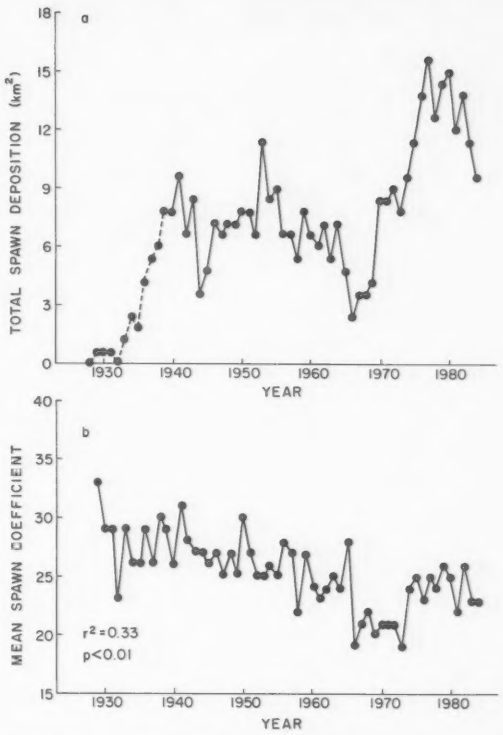

FIG. 4. (a) Total annual British Columbia spawn deposition based on the spawn index. The data prior to 1940, shown by a broken line, may be incomplete. (b) Annual changes in the mean spawn coefficient ($r^2 = 0.33$, $p < 0.01$).

again at the section level. To be most sensitive to geographic variation the spawn coefficient should be calculated and integrated over the smallest possible units; therefore, the locations would be the most desirable units. Many spawn records, however, made during the early years of spawn surveys had incomplete width and intensity estimates. Also, every year some new spawning locations are usually added to the list and some of the geographical names have tended to be replaced by others. Consequently, of the 1114 locations documented as having received spawn, 50–60 have insufficient data for the computation of a spawn coefficient. Spawns in such locations usually are small and their exclusion does not substantially alter the total spawn deposition estimates. Applying and integrating the spawn coefficient at the level of the section (Fig. 1) overcomes these problems, and we have chosen the section as the most appropriate geographical unit over which to apply the spawn coefficient. In effect, the entire coast is divided into approximately 110 sections and the spawn coefficient is calculated for each of the sections. The spawn deposition over any larger geographical unit is the sum of the annual spawn deposition in the small unit.

The greatest annual spawn deposition in British Columbia occurred from 1975 to 1980; the lowest occurred from 1965 to 1970 (Fig. 4a). Prior to 1940, spawn records were incomplete from many locations and the spawn index for those years could be misleading. The lowest point on the index, after 1940, occurred in 1966 following years of large catches. Prior to

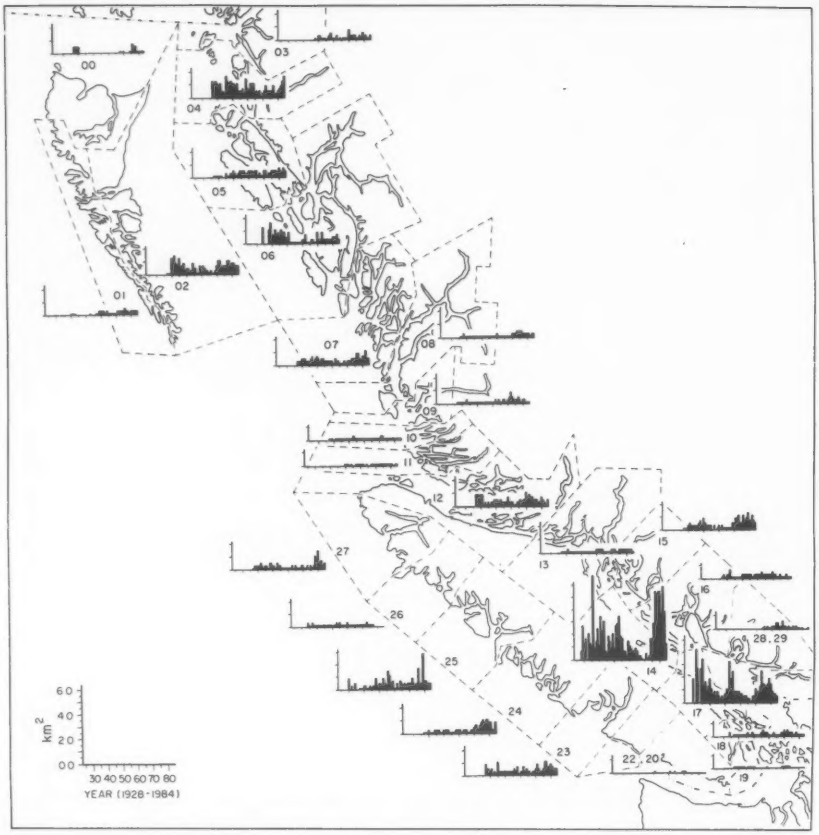

FIG. 5. Geographical distribution of spawn shown for each statistical area. For each area, a histogram is shown of the total spawn area by year.

1965, large catches may have reduced the spawning biomass so that spawn deposition is not a complete indicator of the annual potential spawning stock biomass.

A significant aspect of the spawn coefficient is that when calculated for the entire British Columbia coast, the mean coefficient decreases with time (Fig. 4b) even though the width and intensity estimates for each location are held constant over time. The decrease is significant ($r^2 = 0.33, p < 0.01$). The explanation for this decrease is that the size frequency distribution of spawn records has changed over time: in recent years a larger percentage of the spawn records are made in locations with lower intensities and narrower spawn widths. This is important because it indicates that much of the apparent change in the spawn data is not generated by inconsistent Fishery Officer observations. On the contrary, Fishery Officer estimates of mean width and intensity most often are very consistent for specific locations among years. (It is conceivable that some Fishery Officers could be biased to report similar results among years, but this is very unlikely because others tend to relocate frequently at different sites.) Rather, the change in the mean spawn coefficient reflects a disproportional increase in the relative number of low-intensity, narrow-width spawn records, particularly from northern British Columbia, which generally has narrower spawn widths. No systematic decrease in the mean spawn coefficient occurs when it is examined for smaller geographical units, such as for each statistical area (Fig. 5).

Factors Affecting Spawn Deposition

Biomass of Spawning Stock

To determine whether total spawning biomass affected the spawn dimensions, mean lengths, widths, and intensities were compared with an independent estimate of biomass, calculated only from analyses of age structure (Haist et al. 1985). Estimates of spawning biomass were made from five major divisions of the British Columbia coast. Annual mean estimates of spawn length, width, and intensity were compared, by analyses of regression, with annual spawning biomass estimate. As shown above, however, there are strong time trends in estimates of mean width and intensity which can be attributed to methodological changes in the way the data were gathered. There also is a time trend in the magnitude of catches, which are smaller in recent years. Consequently, spawning escapement usually is greater in recent years. Therefore, to separate and evaluate the effects of biomass and the time trends, multiple regressions of spawn dimensions were compared, in steps, with time (year) and biomass. Then the variation attributable to biomass was evaluated (Table 3).

TABLE 3. Comparison of mean spawning lengths, widths, and intensities with an independent estimate of spawning biomass and year (between 1951 and 1984). Multiple regression examines both factors, biomass and year, simultaneously. For each comparison, the coefficient of determination (R^2) and the F statistic are shown. Degrees of freedom were 1,33 and 2,33, respectively, for the single and multiple regressions. Significance is indicated by a single asterisk for $p < 0.05$ and by two asterisks for $p < 0.01$. The multiple regression shows two F statistics: (1) one for the significance of the multiple regression and (2) $F_{(B)}$ which indicates the degree to which the multiple regression can be attributed to biomass. Degrees of freedom are 2,31 for the multiple regressions and 1,31 for the $F_{(B)}$ statistics. The Queen Charlotte Islands correspond approximately to Statistical Area 2, the north includes all of Statistical Areas 3–5, and the central coast includes most of Statistical Areas 6–8. The west coast includes all of the west coast of Vancouver Island; the Strait of Georgia includes Statistical Areas 14–18, 28, and 29.

		Biomass ×			Year ×			Biomass × year ×		
		mean length	mean width	mean intensity	mean length	mean width	mean intensity	mean length	mean width	mean intensity
Queen Charlotte Islands	R^2	0.0	0.9	4.8	19.0	8.3	43.0	21.5	13.0	43.2
	F	0.0	0.3	1.5	7.5**	2.3	22.1**	4.2*	2.3	10.8**
	$F_{(B)}$							1.0	0.6	0.1
North coast	R^2	1.2	5.0	4.8	37.5	1.4	39.4	45.9	8.5	46.0
	F	0.0	0.0	1.7	19.2**	0.5	19.7**	13.2**	1.4	12.5**
	$F_{(B)}$							1.8	0.0	4.9*
Central coast	R^2	22.6	13.3	23.0	75.8	33.3	68.0	76.8	33.0	68.0
	F	9.3**	4.8**	9.0**	9.9**	15.9**	62.8**	51.2**	7.2**	31.4**
	$F_{(B)}$							1.3	0.0	0.5
Strait of Georgia	R^2	2.7	0.0	3.8	13.4	11.1	19.6	18.9	11.6	32.2
	F	0.9	0.0	1.1	4.9*	4.0	8.3**	3.6	2.0	6.6**
	$F_{(B)}$							2.1	0.2	4.1
West Coast of Vancouver Island	R^2	15.0	29.9	39.7	15.2	44.2	10.8	20.0	50.0	40.1
	F	5.6*	13.7**	20.2**	5.8*	25.4**	3.7*	6.0	17.9	7.4
	$F_{(B)}$							0.4	0.1	14.8**

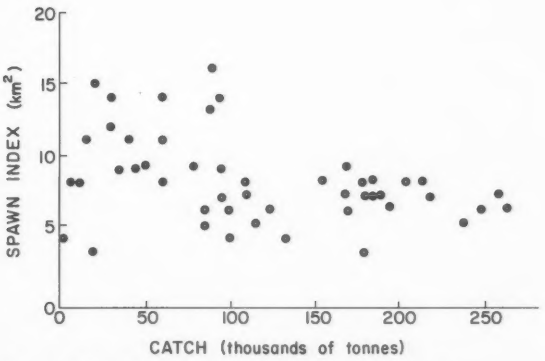

FIG. 6. Effect of catch on total spawn deposition. During years with large catches, spawn deposition is lower; during years with smaller catches, spawn deposition is greater.

TABLE 4. Correlation between total annual spawn deposition and sea-surface temperature in the same and previous years.

Spawn index in year N × temperature in year:	r	p
N	0.013	NS
$N-1$	0.019	NS
$N-2$	−0.359	<0.05
$N-3$	−0.360	<0.05
$N-4$	−0.436	<0.01
$N-5$	−0.315	<0.05
$N-6$	−0.169	<0.05
$N-7$	−0.101	NS
$N-8$	−0.169	NS
$N-9$	0.055	

Of the five geographical divisions examined, none show any significant effect of biomass on mean spawn length or width (Table 3) although all divisions show strong time trends in one or more of the spawn dimensions. In two areas, the north coast and the west coast of Vancouver Island, we found a statistically significant effect of biomass on spawn intensity. Although significant, the effect of biomass on intensity in the north coast is slight, accounting only for about 5% of the variation in intensity. Also, there is a nearly significant correlation of biomass on spawn intensity in the Strait of Georgia, but again the effect is slight. However, the biomass has a strong effect on spawn intensity on the west coast of Vancouver Island. There, the effect of biomass is more pronounced than the time trend and could account for nearly 40% of the variation in spawn intensity. One explanation for changes in intensity is that during years with high biomass, herring may spawn in locations which promote higher intensity. Another, simpler explanation is that in some locations, spawn deposition varies directly as a function of biomass. Higher intensity might be expected if spawning habitat were limited, thus forcing herring to confine spawning to restricted areas. For the coast at large, it seems that there is ample spawning habitat; in a single year, herring spawn in only a small proportion of the total area that has ever been used (Hay 1985). However, it is possible that under certain tidal conditions, and other factors, spawn dimensions could depend on the concentration, size, and density of prespawning aggrega-

TABLE 5. Multiple regression equations relating spawn (km^2) to catch (tonnes) and sea-surface temperature (°C). The best fit occurs when spawn and catch are regressed to temperatures 4 yr earlier.

Temperature lag (yr)	Equation	F	r^2
0	Spawn = 5.67−0.0251 catch−0.0116 temp	3.59	14.0
1	Spawn = 3.92−0.0254 catch−0.0145 temp	3.48	14.0
2	Spawn = 11.4−0.0237 catch−0.0936 temp	7.07	24.9
3	Spawn = 12.2−0.0229 catch−0.1040 temp	6.80	24.9
4	Spawn = 14.7−0.0241 catch−0.1400 temp	9.56	32.3
5	Spawn = 12.6−0.0262 catch−0.1100 temp	6.69	25.5
6	Spawn = 9.09−0.0256 catch−0.0595 temp	4.05	17.6
7	Spawn = 7.83−0.0271 catch−0.0414 temp	4.05	17.9
8	Spawn = 6.65−0.0267 catch−0.0243 temp	3.73	16.9

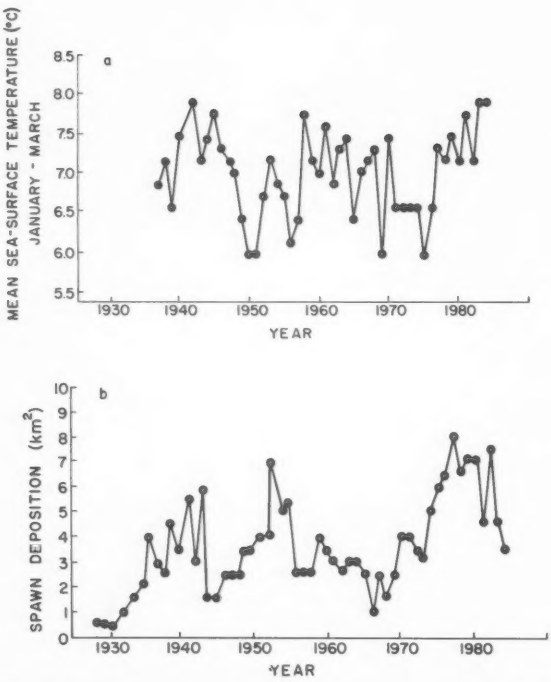

FIG. 7. (a) Temporal pattern in sea-surface temperature in the Strait of Georgia; (b) temporal pattern in total spawn deposition within the Strait of Georgia.

tions (discussed later).

A significant correlation between spawning biomass and spawn intensity introduces bias into the spawn index, which holds intensity, at each section, constant over time. Consequently, the index will tend to underestimate dense spawns and overestimate light spawns — or appear to "dampen" the true oscillations. However, this will be significant only for the west coast of Vancouver Island.

Fisheries

Herring catches have been mainly of maturing fish during winter and spring months, prior to spawning. The removal of large proportions of the population results in a substantial reduction in the total spawn deposition in years with large catches (Fig. 6). The reduction in the spawn index, examined by analysis of regression, is statistically significant ($p < 0.01$). Large catches also might alter geographic spawn distributions, especially if the fishery were geographically concentrated. This is possible in recent years when roe fisheries have occurred during spawning time in specific locations (Hay et al. 1984). Also, removal of fish from the spawning grounds might alter any density-dependent factors controlling spawn deposition parameters by reducing the biomass available to spawn in a limited area.

A Relationship Between Temperature and Spawn Deposition

Within the Strait of Georgia the annual sea-surface temperature records roughly show three cycles (Fig. 7a). The temperature estimates are based on mean of daily records taken from January 1 and March 31 for each year. The data were collected at Entrance Island in the Strait of Georgia. Data until 1972 are from Hollister and Sandnes (1972). Data collected since 1972 were provided by Al Dodimead of the Pacific Biological Station. Spawn deposition also shows three cycles, with maximum depositions occurring during the early 1940s, late 1950s, and late 1970s (Fig. 7b) but spawn and temperature are out of phase by several years. The correlation coefficient between the sea surface temperature and spawn deposition in subsequent years is significant for lag times of 2−5 yr (Table 4). A lag of 4 yr provides the most significant negative correlation between temperature and subsequent spawn deposition. The combined effects of the catch and sea-surface temperature were examined by multiple regression (Table 5). The best least squares fit occurs with a 4-yr lag in the temperature so that total spawn deposition is most sensitive to sea-surface temperatures 4 yr *prior* to the spawn. The probable explanation for this is that temperature must affect, or be strongly correlated with, another factor that affects recruitment and year class strength (Stocker et al. 1985). It appears to take several years for favourable (or unfavourable) temperatures to have an effect on subsequent spawn deposition. Although highly significant ($p << 0.01$), the total effect is still relatively small: the combined effect of catch and temperature does not explain more than 32% of the total variance.

Interactions Between Adjacent Spawning Areas

One explanation for changing distribution of spawns between years is that individual herring may spawn in different

TABLE 6. Annual index of spawn deposition expressed in thousands of square metres among the major geographical divisions of the British Columbia coast. These estimates are not intended to be absolute estimates of area; rather they are relative estimates for comparisons among areas and between years.

Year	Queen Charlotte Is. (Areas 0–2)	North coast (Areas 3–5)	Central coast (Areas 6–11)	Strait of Georgia (Areas 12–19, 28, 29)	West coast of Vancouver Is. (Areas 23–27)	Total coast
1937	208	993	509	3107	867	5 684
1938	208	1174	1179	2679	525	5 765
1939	208	986	1362	4551	809	7 916
1940	1113	1109	962	3470	1107	7 761
1941	916	851	1211	5648	802	9 428
1942	786	834	1503	3029	563	6 715
1943	573	186	1216	6049	501	8 525
1944	449	378	1115	1354	426	3 722
1945	705	419	760	1453	1746	5 083
1946	381	1329	1683	2680	975	7 048
1947	593	1060	1321	2556	783	6 313
1948	797	1328	1405	2429	1526	7 485
1949	271	1447	871	3649	1026	7 264
1950	282	1557	1151	3564	1409	7 963
1951	244	1143	995	3901	1355	7 638
1952	196	768	441	4200	742	6 347
1953	410	1060	857	7159	1933	11 419
1954	717	913	598	4800	1394	8 422
1955	418	893	687	5562	1443	9 003
1956	467	991	504	2743	1785	6 490
1957	110	1456	300	2364	2291	6 521
1958	68	514	596	2485	1544	5 207
1959	613	1378	591	4219	959	7 760
1960	317	1123	874	3502	772	6 588
1961	710	1035	438	3127	823	6 133
1962	437	1598	1381	2658	1327	7 401
1963	338	829	511	3037	923	5 638
1964	329	1259	680	3241	1592	7 101
1965	128	517	341	2312	1508	4 806
1966	230	361	295	1137	552	2 575
1967	79	234	613	2302	563	3 791
1968	152	454	575	1638	648	3 467
1969	155	77	300	2436	992	3 960
1970	385	905	1170	4162	1755	8 377
1971	652	737	1018	3999	1850	8 256
1972	711	1218	1330	3593	2127	8 979
1973	699	757	1825	2936	1285	7 502
1974	1151	697	1388	5103	1011	9 350
1975	884	851	1530	5961	2014	11 240
1976	1616	1174	2111	6319	2837	14 057
1977	1909	1498	2005	7823	2294	15 529
1978	1760	760	1138	6419	2494	12 571
1979	1360	832	1056	6755	4162	14 165
1980	1867	1572	1578	6901	3014	14 932
1981	1980	1198	1842	4722	2071	11 813
1982	1916	1373	1606	7362	1827	14 084
1983	1356	1901	2011	4569	1277	11 114
1984	1340	2130	1508	3368	1126	9 472

places in different years. For instance, it is conceivable that environmental conditions or other factors could promote spawning within the Strait of Georgia one year and somewhere else, such as the west coast of Vancouver Island, in another year. If herring spawns did shift among areas, then an inverse relationship in spawn deposition might be expected: when spawn deposition is high in one area, it should be low in another.

To test this, the total spawn deposition was summed annually for each major geographic "division" (Table 6). Of the 10 comparisons of annual spawn deposition among major areas examined, all are positive and 8 are significant ($p < 0.05$) (Table 7). This indicates that periods of abundant spawn deposition among different regions are positively correlated. Therefore, a decrease in spawn deposition within a division, such as the Strait of Georgia, is not necessarily explained by a mass movement of fish to other areas. This conclusion, however, does not necessarily apply to smaller geographical scales. The same correlation analysis, when applied among statistical areas within the Strait of Georgia, often shows negative correlation between some statistical areas (Table 8). For instance, there are significant negative correla-

TABLE 7. Correlation matrix of the spawn deposition compared among major geographical divisions of the British Columbia coast from 1928 to 1984. Single and double asterisks indicate significance at the 0.05 and 0.01 probability levels, respectively.

	Queen Charlotte Is.	North coast	Central coast	Strait of Georgia
North coast	0.379**			
Central coast	0.682**	0.463**		
Strait of Georgia	0.667**	0.256	0.451*	
West coast of Vancouver Is.	0.558**	0.257	0.285*	0.633**

tions between spawn deposition in Area 14 and each of Areas 16, 18, 28, and 29. There are, however, positive correlations between other areas. Throughout the history of spawning in the Strait of Georgia, some areas such as Statistical Area 14 receive herring spawn intermittently. When this occurs, it seems to cause a decrease in spawn abundance in adjacent areas. Aside from Area 14, there are generally positive correlations for annual spawn deposition among other areas. This result is consistent with conclusions about stock structure based on tagging data by Hourston (1982) who estimated that 77–94% of herring "homed" or where recovered within the same geographical division in which they were tagged. The divisions used in the tagging analysis were identical to those in Table 6 except that Johnstone Strait (Statistical Areas 11 and 12) was separated from the Strait of Georgia. Lower "homing" estimates were made on smaller geographical units. Similarly, the correlation matrices of the annual spawn index indicate that there is no evidence of shifts of spawn (indicated by negative correlations) between the large geographical divisions, but there is evidence of shifts among smaller, adjacent areas. A discussion of possible movements of herring among spawning locations will be discussed elsewhere.

Density-Dependent Effects on Spawn Distribution

Prespawning aggregations of herring usually congregate in the vicinity of a spawning beach several days prior to spawning. The first spawning appears to be initiated by males; females move in slightly later (Hay 1985). Large, long spawnings tend to begin at one end or at several different sites and proceed along the shore as spawning progresses. The duration of larger spawnings can be 3 d or longer. During the spawning there must be some mechanism, perhaps a biological feedback, which acts to limit egg density and promote the movement to fresh spawning sites along the beach. Such a feedback mechanism might operate through milt density (Hay 1985) or through fish density. Aerial observations of spawnings indicate that fresh or current centers of spawning activity can often be distinguished against the background of older milt. These small fresh milt deposits are evidence that very large spawnings may be accumulations of much smaller, discrete spawning acts. A large single spawn deposition may be many kilometres long and involve thousands of tonnes of fish, but it appears that the actual milt deposition and egg release occur in smaller units (schools). For instance, it would sometimes be physically impossible for one very large school of herring to deposit all of its eggs at one geographical focus. The potential spawn width and intensity are limited by the topography and surface area or foliage of the submergent and intertidal vegetation: steep-sided locations with narrow bands of sparse vegetation tend to have narrow, low-intensity spawn deposition. In contrast, wide locations with dense vegetation have the potential for wide, high-intensity spawn deposition.

The lateral movement of a spawn along a shoreline could occur both as a result of tidal movements or currents and perhaps biological factors such as fish density or concentration of milt (Hay 1985). Spawning usually does not occur in the immediate vicinity of heavy surf activity, which often is found on open coasts. In these circumstances, spawning is confined to the protected sides of islands or even to completely submerged shallow reefs.

Within very narrow locations (i.e. <5 m), maximum spawn width nearly always would be achieved quickly, even with small schools. Within wide locations, it would take longer to reach the maximum width; possibly lateral movement along the shore could occur before the maximum spawn width occurred. This can be illustrated as follows. Suppose the average intensity of spawning were constant at 500 000 eggs/m^2 (a density commonly observed; Haegele et al. 1981). Then a 1000-t aggregation would deposit approximately 10^{11} eggs based on an average relative fecundity of 10^8 eggs/t (Hay 1985). A total area of 2×10^5 m^2 (i.e. $10^{11}/(5 \times 10^5)$) would be used for spawning. The theoretical length of this spawn in a location with a width of 10 m would be 2×10^4 m or 20 km (i.e. 2×10^5 m^2/10-m width). The normal duration of a spawn of this magnitude might be roughly 2 d. If spawn deposition occurred evenly over these 48 h, then the expected area coverage would be roughly 2×10^5 m^2/48 h or 4200 m^2/h (about 70 m^2/min). However, it is probable that this deposition rate would occur more slowly at the beginning, followed by acceleration as spawning activity reached a peak, followed again by deceleration as the spawning was completed. In many regions of the coast, the tidal velocity normally reaches 4–5 km/h (2–3 knots) or more. Spawning herring can swim sufficiently well to stem this tidal velocity, but sperm (or milt) in the water could not. To ensure fertilization, females must remain in the vicinity of milt and, presumably, follow the tides. Therefore, at maximal tidal velocity, milt-containing water should move over the spawning grounds at 60–80 m/min or about 1 m/s. If a spawning location had a width of 100 m and a 1000-t school could deposit eggs at an average rate of 70 m^2/s, then a full spawn width of 100 m might not be achieved. In a location with a width of only 10 m, a 1000-tonne school could utilize seven times the maximal width and would require more than one center of spawning activity. Although these estimates are rough, they illustrate that within specific locations, spawn widths could depend on school size, tidal velocity along the shore, and the time of spawning within the tidal cycle.

Applications and Implications for Management

Within industry and government, there have been concerns that the recent (post-1970) roe fishery may be causing "shifts" of spawn, mainly from the south to the north. The analyses in this paper do not support such concern: in general, spawn deposition tends to be positively related between different major regions. There have also been concerns that the stocks have not fully recovered since the drastic decline of the late 1960s. Our results indicate that total spawn deposition in recent years is at least equal and perhaps greater than those of earlier (1930–60) years. Schweigert and Stocker (1987) reached a similar conclusion based on their work which

TABLE 8. Correlation matrix of spawn deposition within the Strait of Georgia. The annual spawn deposition in each statistical area is compared with each of the other areas. Statistical Areas 19, 28, and 29 are pooled. Significant positive and negative correlations are noted with one or two asterisks indicating 0.05 and 0.01 levels of significance, respectively.

	Area						
Area	12	13	14	15	16	17	18
13	0.328*						
14	0.051	0.247					
15	0.568**	0.347*	0.409*				
16	0.323*	0.340*	−0.307*	0.060			
17	0.151	0.024	0.208	0.153	−0.068		
18	0.509**	0.188	−0.308*	0.401**	0.440**	0.043	
19 + 28 + 29	−0.085	0.305	−0.309*	−0.128	0.289	−0.234	0.131

attempts to derive an absolute estimate (tonnes) of spawning biomass. Yearly variation in the absolute biomass estimate is closely correlated with the relative spawn index used in this paper, with a correlation coefficient of 0.96 for the British Columbia coast as a whole. To derive their estimate of tonnes of spawners, they compared Fishery Officer spawn data with that collected by SCUBA diving surveys. Correction factors were derived to convert egg intensity to the more recent "egg layers," and they developed a correction factor to adjust for the possible underestimate of widths by Fishery Officers. The correction factors are derived from and applied to large geographic areas, such as the entire west coast of Vancouver Island or the Queen Charlotte Islands, but finer geographic adjustments may be possible following future work in this topic. In the absence of SCUBA diver observations on spawn deposition, a relative spawn index, like the one used here, should be suitable for monitoring temporal changes in spawning abundance even within relatively small geographical areas such as a "section" or "statistical area" (Fig. 1). From the time trend in this index, it is clear that total spawn deposition from 1980 to 1984 exceeded that of the period from 1940 to 1965, when a major reduction fishery was conducted. By 1967, following years of very high catches, the stocks collapsed, but in the early 1970s they had apparently recovered sufficiently to begin a new roe fishery. It is not clear, however, if the total prefishery stock levels throughout the coast during the current roe fishery are as great as those made during the earlier reduction fishery. Very large catches of mature and immature fish during the reduction fishery make the issue complex. There is no question, however, that spawn deposition has fallen sharply since 1983. This decline, which may have begun before 1983, occurred in southern British Columbia areas and Washington State (Penttila 1986). Also, spawns have declined in many areas that have not been exposed to roe fisheries. The explanation for this decline is unclear but is probably related to unfavourable temperature regimes in the early 1980s (D. Ware, Pacific Biological Station, pers. comm.). This contention is supported by the results of this paper which show that spawn deposition is significantly correlated to January–March temperatures 3–6 yr earlier.

Herring spawn surveys have been, and continue to be, an essential and integral part of the herring stock assessment and management process. Since 1984, the spawn index presented here has been included in that process. On the west coast of Vancouver Island, low spawn deposition in 1984 and 1985, in addition to other criteria, led to fishing closures in both of the following years, 1985 and 1986. In the Strait of Georgia, a low spawn deposition in 1984 and 1985 led to a reduction of the 1985 fishery and a closure in 1986. These low spawn depositions, indicative of a reduction of spawning stock size, have been corroborated by other assessment methods including age-structure analyses (Haist et al. 1986) and hydroacoustic surveys (Armstrong 1986). Decisions concerning quotas, fishery openings, and closures are made after a thorough review of stock assessment and related analyses as well as input from Fishery Officers throughout the coast (Haist et al. 1985, 1986).

In contrast with the low spawn deposition in southern British Columbia and Washington State (Pentilla 1986), central and northern British Columbia stocks in the mid-1980s have exceptionally high spawn deposition, in some cases exceeding previous records (Haist et al. 1986). However, there was a substantial improvement in 1986 spawning in southern waters, and fisheries for 1987 are planned for all locations on the British Columbia coast. The continued effective management of this fishery will require the continuation of annual spawn surveys into the foreseeable future.

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Diel Activity and Foraging Behavior of Juvenile American Lobsters, *Homarus americanus*

Peter Lawton[1]

Department of Fisheries and Oceans, Fisheries Research Branch, Invertebrates and Marine Plants Division, Biological Station, St. Andrews, N.B. E0G 2X0

Lawton, P. 1987. Diel activity and foraging behavior of juvenile American lobsters, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 44: 1195–1205.

The activity of wild-caught juvenile American lobsters, *Homarus americanus* (20–46 mm carapace length (CL)), was monitored in the laboratory using time-lapse video recording techniques. Behavioral time-budget analyses indicated that juvenile lobsters are nocturnally active within or close to available shelter. Mussels, *Mytilus edulis*, provided as prey were picked up, apparently nonselectively, when lobsters ventured out from shelter and were immediately taken back to shelter for consumption. Such shelter-related or central-place foraging behaviors may minimize risk from predation during transition to the wide-ranging activity pattern typical of larger lobsters. Increased hunger led to greater light-phase activity, primarily shelter-related foraging behavior, among individually monitored lobsters. Dominant lobsters in CL-matched pairs also exhibited this behavior pattern, but spent less time within shelter and feeding during dark phases due to involvement in agonistic interactions. Subdominant lobsters spent the least time in shelter, often remained inactive in open areas of tanks, and foraged predominantly during light phases. Increase in predation risk due to adoption of these behaviors may be the major consequence of subdominant status rather than a reduction in food intake.

L'activité de homards (*Homarus americanus*) juvéniles de 20 à 46 mm de longueur de carapace (LC) capturés à l'état sauvage a été contrôlée au laboratoire à l'aide de techniques de surveillance vidéo image par image. Des analyses temporelles du comportement ont montré que les homards juvéniles étaient actifs la nuit, sous ou près de la couverture disponible. Des moules *Mytilus edulis*, servant de proies, étaient capturées, de façon non sélective, par les homards lorsque ceux-ci s'aventuraient à l'extérieur de leurs abris. Elles étaient ensuite immédiatement ramenées dans les abris pour être consommées. Un tel comportement de recherche de nourriture lié à l'abri ou à un point central pourrait permettre de minimiser les risques de prédation au cours de la période de transition qui mène au très large régime d'activité typique des gros homards. On a noté, chez des homards surveillés individuellement, que l'augmentation de la faim se traduisait par une plus grande activité, surtout un comportement de recherche de nourriture lié à l'abri, en conditions d'éclairement. Des homards dominants appariés par LC présentaient aussi ce mode comportemental mais passaient moins de temps dans les abris ainsi qu'à se nourrir en périodes d'obscurité à cause d'interactions du type antagoniste. Les homards sous-dominants étaient ceux qui passaient le moins de temps dans les abris, restaient souvent inactifs dans des zones dégagées des réservoirs et recherchaient surtout leur nourriture qu cours des phases d'éclairement. L'augmentation des risques de prédation découlant de ces comportements, et non la réduction de la consommation de nourriture, pourrait être la principale conséquence de la sous-dominance.

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The American lobster, *Homarus americanus*, is the object of important commercial fisheries in the Canadian Maritimes (Campbell and Mohn 1983) and northeastern United States (Dow 1980). Considerable information is available on diel activity and movement patterns of adult lobsters (Weiss 1970; Stewart 1972; Cobb 1977; Cooper and Uzmann 1980; Ennis 1983; Richards et al. 1983). Comparable data are lacking for juvenile lobsters, and this has compromized attempts to elucidate the ecology and population dynamics of early life-history stages (Bernstein and Campbell 1983).

Following settlement to the bottom, postlarval lobsters are able to burrow into a variety of substrates (Cobb 1971; Berrill and Stewart 1973; Atema et al. 1982; Pottle and Elner 1982). They adopt a cryptic life style in which tunnel systems may provide both food and shelter for the first 2 yr of benthic existence (Cooper and Uzmann 1980; Atema et al. 1982). Early juvenile stages (<35 mm carapace length (CL)) are difficult to locate in the wild; much of our information on their activity pattern is derived from hatchery-reared stock (Cobb 1971; Zeitlin-Hale and Sastry 1978; Atema et al. 1982; Pottle and Elner 1982; Roach 1983) or by inference from studies with larger lobsters (Jacobson 1977; O'Neill and Cobb 1979; Richards 1984).

SCUBA diver observations in natural lobster habitats suggest that wide-ranging activity, typical of adult lobsters, develops over the 35–50 mm CL range (Weiss 1970; Cooper and Uzmann 1980; Ennis 1983). At this size, juvenile lobsters are exposed to considerable predation risk which remains until defensive behaviors become fully developed (Lang et al. 1977) and a larger size is reached (Cooper and Uzmann 1980).

[1]Present address: Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000, USA.

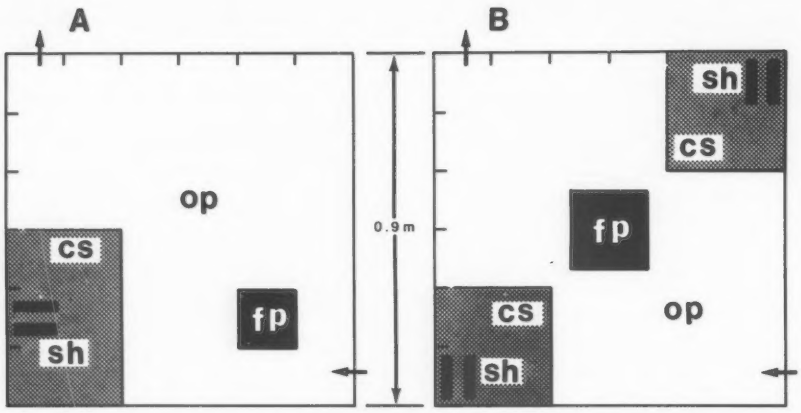

FIG. 1. Classification of bottom area of tanks used in activity monitoring experiments into shelter (sh), area close to shelter (cs), the open (op), and the food patch (fp). Bold arrows denote location of seawater inflow and outflow.

Hunger level and intraspecific competitive interactions are potentially important factors which may influence diel activity and foraging behavior, and thus susceptibility to predation (Morse 1980). These factors have not been fully explored in previous studies on juvenile lobsters (Jacobson 1977; Carter and Steele 1982; Cobb et al. 1982). In the present study, the diel activity of wild-caught juvenile lobsters of 20–46 mm CL was monitored in laboratory environments as they foraged upon a preferred sessile epifaunal prey, the blue mussel, *Mytilus edulis* (Elner 1980; Carter and Steele 1982). Time-budget analysis, an important approach to the study of behavioral strategies (Hanley 1984), was utilized to characterize the activity pattern of recently captured lobsters and to examine behavioral plasticity in response to variation in hunger level and degree of social interaction.

Materials and Methods

During August–October 1983–84, juvenile lobsters were collected by SCUBA divers from nearshore waters of 3.0–10.0 m depth at McNutts Island, southwestern Nova Scotia (43°38′N 65°18′W), and transported to the Department of Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick. The CL (millimetres), wet weight (WW, grams), sex, and molt condition (Aiken 1973) of each lobster were recorded upon arrival. CL was measured from the base of the eye socket to the posterior margin of the carapace parallel to the mid-dorsal line. Large lobsters (>40 mm CL) were then placed singly into 0.6 × 0.4 m aquaria filled with running seawater (0.2 m depth). Plexiglas partitions, having opaque lower borders (0.1 m depth), subdivided some aquaria into either two or four compartments. These aquarium compartments were used for holding smaller lobsters (<40 mm CL) and for conducting preliminary feeding experiments. Shrimp (*Pandalus borealis*), crab (*Cancer irroratus*, *Carcinus maenas*), fish (*Clupea harengus*), or blue mussel body tissues were used as maintenance foods, depending on availability, and were presented ad libitum twice weekly. Individual lobsters were retained for up to 26 wk but were only used in experiments if they still had intact claws and antennae and were in the intermolt stages of C_4–D_0 (pleopod stages 0–2.0; Aiken 1973). Mussels obtained from the mid-littoral zone at Brandy Cove, St. Andrews, and cleaned of byssus filaments were used as prey. Shell length (SL, millimetres) of mussels was measured along the anterio-posterior axis parallel to the ventral surface of the shell.

Mussels as Prey

The critical size (sensu Vermeij 1976) of mussel susceptible to predation by a lobster of a given CL was defined as the mean of the SL of the largest mussel broken and the SL of the smallest mussel resistent to breakage. Five male and five female lobsters (24–42 mm CL) were individually presented with four small mussels placed on the floor of the aquarium compartment; eaten mussels were replaced daily with larger individuals until 5 d elapsed without further predation.

The amount of mussel flesh consumed by juvenile lobsters after each of six starvation periods (6, 12, 18, 24, 48, and 72 h) was determined using 13 male and 7 female lobsters (22–50 mm CL). Sixteen trials were conducted at each hunger level by first presenting mussel flesh ad libitum to a lobster and confirming satiation 45–60 min later when food was ignored or actively rejected. After the experimental starvation period, preweighed (milligrams WW) mussel flesh was presented for 60 min until satiation was again evident. Two preweighed (milligrams WW) mussel flesh samples placed in an adjacent aquarium compartment at the start of the latter presentation acted as a control for mussel flesh loss due to handling procedures. Flesh consumption (milligrams dry weight, DW) adjusted for loss was derived by collecting control samples and uneaten flesh and drying these tissues for 48 h at 60°C.

Mussel flesh consumption during activity monitoring experiments was estimated by recording the SL of eaten mussels, applying a $\log_e$–$\log_e$ regression of flesh DW on mussel SL (derived from measurement of the flesh DW of mussels 5–37 mm SL), and then subtracting the DW of flesh remaining attached to broken shell valves.

Activity Monitoring

Lobster activity was monitored, with a closed circuit video time-lapse system, in four 0.9 × 0.9 m fibreglass tanks filled

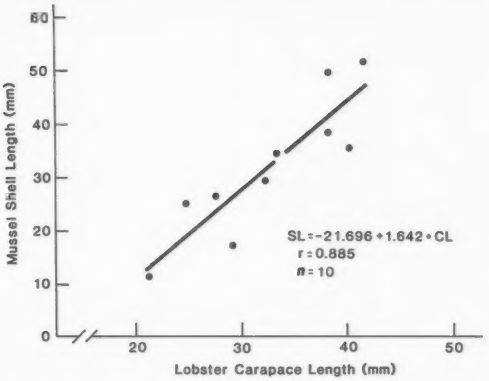

FIG. 2. Critical size (shell length) of mussels vulnerable to predation by juvenile lobsters of a given carapace length.

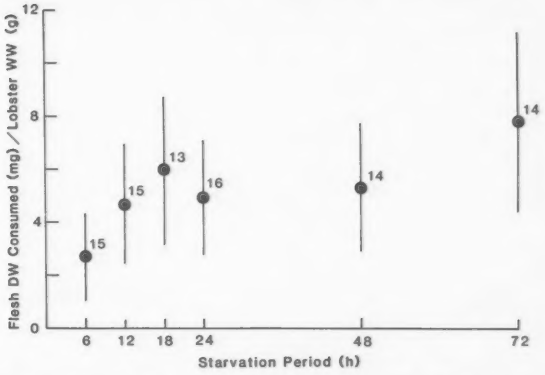

FIG. 3. Amount of mussel flesh consumed by juvenile lobsters of 22–50 mm CL (9–101 g WW) after each of six starvation periods. Data are means ± SD. One observation of no feeding after 6 h and seven observations where consumption was <1.5 mg flesh DW/g lobster WW after longer starvation periods were removed, yielding the sample sizes indicated.

to a depth of 0.2 m with running seawater. Illumination during light (L) phases was provided by two timer-controlled fluorescent-light units; two 15-W lamps shielded by filters (Kodak safelight 1A) gave continuous low-level illumination in the near-infrared (>600 nm) and allowed monitoring over dark (D) phases.

Experiment A: Diel activity of recently captured lobsters

Diel activity of five male and five female lobsters (20–46 mm CL) was monitored within 14 d of their initial capture from the wild. Trials commenced at 08:00 (day 1) when a lobster was placed in the center of a tank and observed until it occupied the glazed clay tile provided as shelter (Fig. 1A). Mussel flesh was presented ad libitum at the shelter entrance until 09:00 when remaining flesh was removed and the lobster left undisturbed for 24 h to standardize initial starvation period. Prior to commencement of video recording (47-h duration) at 09:00 (day 2), intact mussels were placed in a defined area of the tank (Fig. 1A). The size-class (SL) and numerical composition of the prey was dependent on lobster CL (see Table 2). At the end of each trial (08:00, day 4), shell debris and intact mussels were examined. Seawater temperature ranged between 12.8 and 16.0°C over the course of the experiment (August–October 1984) and was monitored daily. Photoperiod was adjusted at 4-d intervals using sunrise and sunset times for the latitude and longitude of McNutts Island. Fluorescent lights switched on 30 min prior to sunrise and switched off 30 min after sunset; there were no crepuscular periods.

Experiment B: Effects of hunger level and conspecific presence on activity level

Using an experimental protocol similar to Experiment A, four male lobsters were monitored both singly and as members of two CL-matched pairs (see Table 3) as they foraged on mussels after each of three initial starvation periods (24, 48, and 72 h). One lobster in each paired-lobster trial was marked on the dorsal surfaces of the carapace and claws using white nail varnish. Thus, dominance/subdominance relationships could be followed and individual lobster behavior compared between trials. The size-class and numerical composition of prey placed within the food patch (Fig. 1B) prior to video recording (22-h duration commencing at 10:00) was the same in both single- and paired-lobster trials (see Table 3). Experiment B, conducted in January 1984, was performed over a 31-d period, utilizing lobsters captured in October 1983. Paired-lobster trials commenced between 1 and 9 d following pairing; single-lobster trials commenced between 2 and 13 d after lobster pairs had been separated. Lobsters were maintained under controlled temperature (12 ± 1°C) and photoperiod regimes (12 h L : 12 h D, with the L phase commencing at 08:00) until all treatment combinations had been completed.

Behavioral Analyses

The complete video record of both experiments was reviewed. Locomotor activity occurring in each 30-min period of a trial was scored with reference to the spatial pattern of lobster movement. If the lobster remained within shelter, 0 was scored. A score of 1 was allocated if the lobster exited from shelter within the period, but was only active in an area close to shelter (Fig. 1). Lobsters that were active in the open area of tanks (Fig. 1) received a score of 2. Feeding activity was similarly scored as 1 (present) or 0 (absent) in each 30-min period. In paired-lobster trials (Experiment B), these scores reflected the activity pattern of both lobsters (e.g. 4 was scored if both were generally active).

Recording segments were selected for quantitative analysis of the allocation of time to different activities (time-budgeting). In Experiment A, 30-min segments beginning at noon (12:00) and at midnight (24:00) were chosen for comparison of L and D phase time-budgets. Additionally, 30-min segments at lights-off (15 min prior, to 15 min after the onset of the D phase) and at lights-on (15 min prior, to 15 min after the onset of the L phase) were analyzed. In Experiment B, recording segments of 120 min were selected in the L phase (commencing at 12:00) and in the D phase (commencing at 19:00). These longer recording segments permitted assessment of the effects of social interaction on time-budgeting.

Time-budgets were constructed by summing the durations (seconds) of discrete bouts of the following activity patterns occurring within a defined recording segment: W_{sh} = lobster within shelter (Fig. 1), but not observable; A_{sh} = lobster within

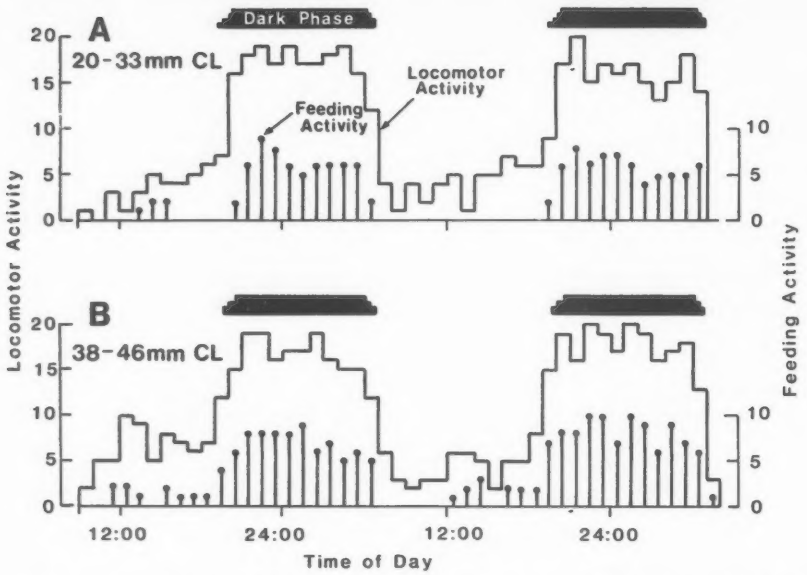

FIG. 4. Diel activity of juvenile lobsters (A) 20–33 mm CL (n = 5) and (B) 38–46 mm CL (n = 5) monitored in Experiment A. Hourly locomotor activity is the sum of individual scores of 0 (remained within shelter), 1 (only active close to shelter), or 2 (active in open) for n lobsters in two 30-min periods. Hourly feeding activity is the sum of individual scores of 0 (not feeding) or 1 (observed feeding) in each 30-min period. The photoperiod (hL:hD) under which each of the n lobsters was monitored was based on the ambient photoperiod at time of capture and thus varied between replicates as shown.

shelter; appendage and body movements observable at entrance, indicative of an alert state; F_{sh} = shelter-related foraging activity including feeding on mussels previously brought to the shelter, and foraging movements out of shelter towards the food patch (Fig. 1) followed by an immediate return to shelter with captured prey; A_{cs} = locomotor activity restricted to an area close to shelter (Fig. 1), typically involving an exit forwards (up to one body length) out of shelter followed by a return backwards into shelter, executed in one continuous movement; A_{op} = general locomotor activity in the open area of the tank (Fig. 1); F_{op} = foraging or feeding on mussels in the open area or within the food patch (Fig. 1); I_{op} = inactive in the open; no locomotor activity or appendage movement apparent for >120 s.

Foraging activity referred to the search for and acquisition of mussels, and incorporated feeding activity. The latter activity was defined as the manipulation of mussels in the claws prior to shell breakage and the consumption of exposed tissue thereafter.

Transitions between individual bouts of the above activity patterns occurred as lobsters left shelter, moved between different areas of the tank (Fig. 1), or commenced (ceased) feeding. Bouts in progress at the beginning or end of a defined recording segment were used in calculating the time-budget and the frequency of activity pattern change, but were not used for determining mean bout durations.

In trials with CL-matched pairs (Experiment B), social interactions were not included explicitly in the time-budget; however, their frequency of occurrence, together with the success or failure of the lobster initiating the exchange, was noted. Three categories were recognized, partially based on previous descriptions of intraspecific agonistic behavior in lobsters (reviewed by Atema and Cobb 1980).

(1) Approach in the open. Movement towards the other lobster by the initiator. The interaction was *won* if avoidance behavior was exhibited by the other lobster and *lost* if the initiator retreated following agonistic displays.

(2) Shelter contest. The initiator approached a shelter occupied by the other lobster. The initiator *won* if occupancy was exchanged and *lost* if the resident defended the shelter successfully.

(3) Disturbance at feeding site. The initiator approached the other lobster while it was feeding (within shelter or in the open) and *won* if it caused the feeding session to be terminated. The initiator *lost* the interaction if the other lobster continued to feed after agonistic display.

Results

Mussels as Prey

Critical size of mussel prey increased linearly with lobster size (Fig. 2). The largest mussel successfully attacked (52 mm SL opened by a 45 mm CL lobster) was close to the maximum size (53 mm SL) encountered at Brandy Cove. Towards the critical size some prey were only partially broken (anteriorly); posterior adductor muscles held both shell valves together, thus reducing further breakage.

The amount of mussel flesh consumed by juvenile lobsters increased over the 6-, 12-, and 18-h starvation periods, there-

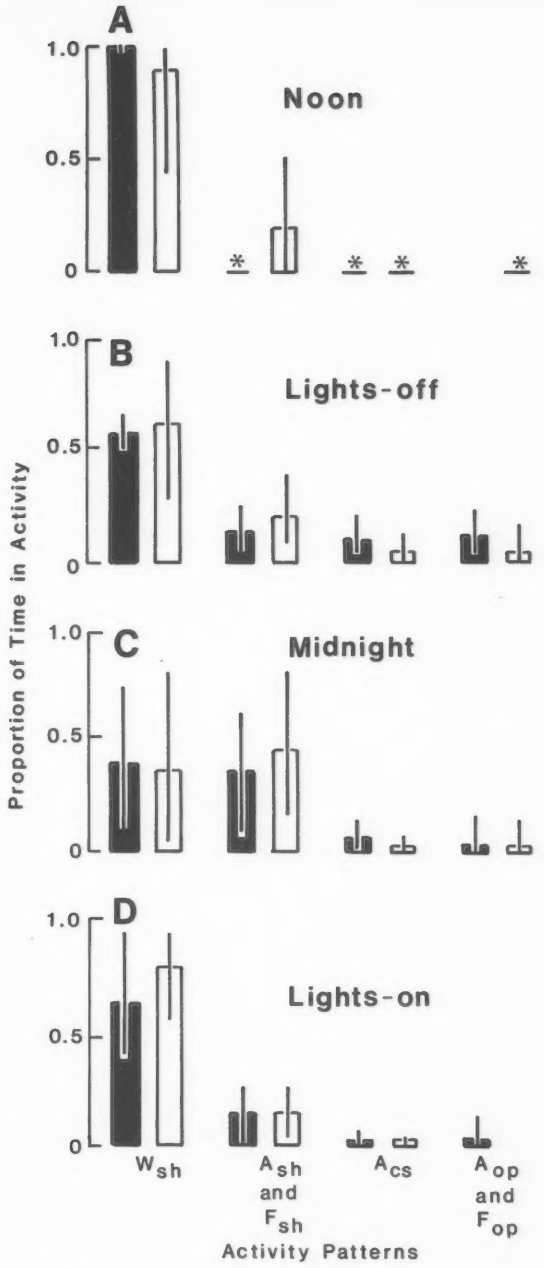

FIG. 5. Time-budgeting over 30-min periods at (A) noon, (B) lights-off, (C) midnight, and (D) lights-on by juvenile lobsters monitored in Experiment A. The mean ± SD proportion of time spent in six activity patterns (derived from arc-sine square-root transformed data, $n = 10$) is indicated for lobsters 20–33 mm CL (solid bars) and 38–46 mm CL (open bars). Time-budgets of individual lobsters for the same period on consecutive days were regarded as independent ($n = 5$ lobsters × 2 d). Refer to the text for explanation of activity pattern acronyms. An asterisk indicates that the activity contributed <0.005 to the time-budget on a proportional basis.

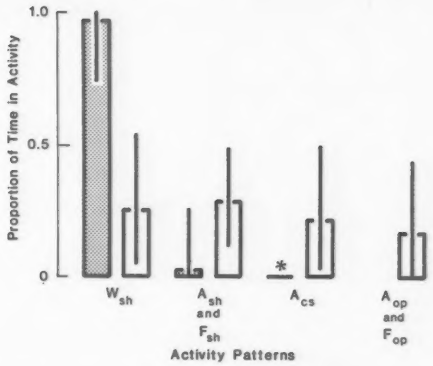

FIG. 6. Time-budgeting by juvenile lobsters at commencement of the D phase (lights-off period). The mean ± SD proportion of time spent in six activity patterns ($n = 20$) is indicated for 10 lobsters (20–46 mm CL) monitored for 15 min before (stippled bars) and for the first 15 min after (open bars) the onset of the D phase. Other plotting details as in Fig. 5.

after reaching a plateau at approximately 6 mg flesh DW/g lobster WW (Fig. 3). Mussel flesh DW was related to mussel SL according to the regression

$$\log_e \text{DW} = -4.715 + 2.804 \log_e \text{SL}$$
$$r = 0.986, \quad n = 80.$$

Flesh consumption, herein presented in terms of flesh DW (milligrams), may be converted to a WW basis (milligrams) using the following relationship:

$$\log_e \text{WW} = 1.459 + 1.045 \log_e \text{DW}$$
$$r = 0.996, \quad n = 80.$$

Diel Activity Pattern of Recently Captured Lobsters

Locomotor and feeding activity indices generated from the complete video record of Experiment A indicated distinct nocturnal peaks to both activities. Large shifts in activity level occurred in the 3 h around each light-cycle change (Fig. 4). However, these indices (based on presence/absence categorization) masked certain trends in activity levels and behavioral differences between the two lobster size groups. Although both types of activity remained elevated through the D phase, a gradual decrease was apparent from approximately 24:00 onwards. Activity of smaller lobsters (Fig. 4A) was closely entrained to the L:D cycle, particularly with regard to feeding activity. The only records of feeding during L phases for this group were of a single lobster (33 mm CL) observed feeding in five consecutive 30-min periods (13:30–16:00 on day 1). Locomotor activity of smaller lobsters within the L phase was typically confined to the area close to shelter. Conversely, four of five larger lobsters (Fig. 4B) fed during both L phases and were also scored as generally active.

The time-budget of juvenile lobsters was dominated by shelter-related activities. At noon (Fig. 5A), small lobsters were primarily recorded W_{sh}. Ten bouts of A_{sh} activity were observed, but these were all <30 s in duration, representing <0.005 of the time-budget on a proportional basis (Fig. 5A) Two A_{cs} bouts occurred, each of which lasted <9 s; there was

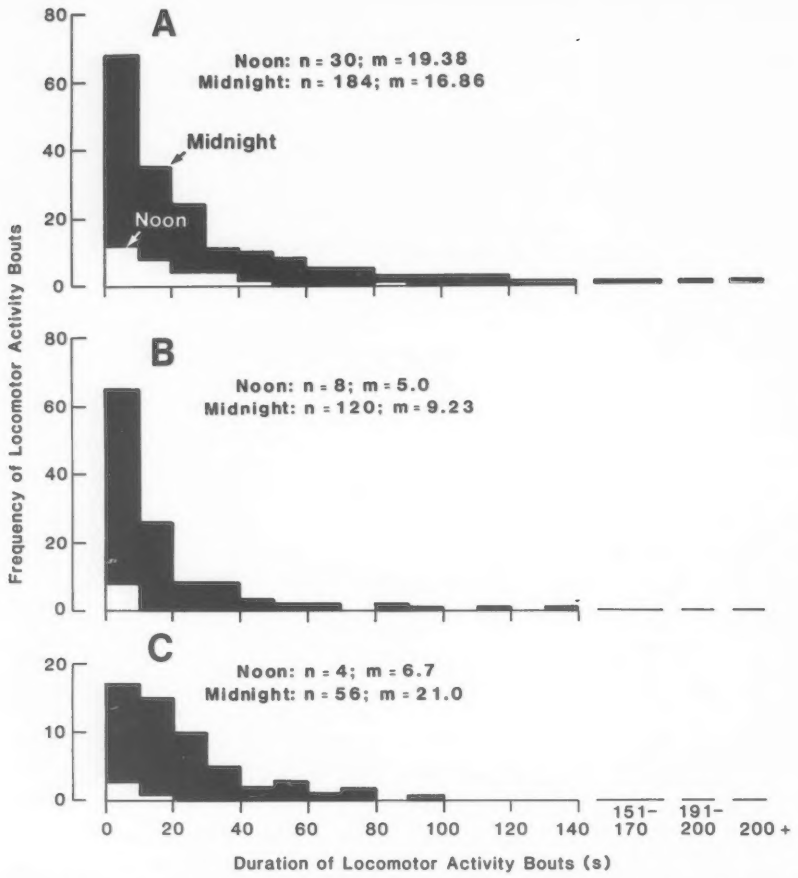
A
Noon: n = 30; m = 19.38
Midnight: n = 184; m = 16.86
Midnight
Noon
B
Noon: n = 8; m = 5.0
Midnight: n = 120; m = 9.23
C
Noon: n = 4; m = 6.7
Midnight: n = 56; m = 21.0
Frequency of Locomotor Activity Bouts
0 20 40 60 80 100 120 140 151-170 191-200 200+
Duration of Locomotor Activity Bouts (s)

FIG. 7. Frequency distribution of the duration of locomotor activity bouts conducted by juvenile lobsters (20–46 mm CL, $n = 10$) monitored for 30 min commencing at noon and at midnight over two L:D cycles within 14 d of capture. (A) Locomotor activity within shelter; (B) locomotor activity close to shelter; (C) locomotor activity in the open. Number of bouts (n) and median bout lengths (m) are indicated.

no foraging activity (F_{sh}, F_{op} patterns). Larger lobsters were observed at the shelter entrance more frequently (21 bouts of A_{sh} up to 84 s in duration). One lobster (42.0 mm CL male) was very active at noon, engaging primarily in F_{sh} activity (19.9 min on day 1, 27.4 min on day 2). The shift in juvenile lobster activity level at commencement of the D phase was due to limited increases in A_{sh} activity prior to the light change and marked increase in A_{cs} and A_{op} activities afterwards (Fig. 5B, 6). Feeding activity was minimal during this period. Overt activity was greatest at midnight, comprising >0.5 of the time-budget, the major contribution arising from a number of prolonged F_{sh} bouts (Fig. 5C). Within-shelter activity (W_{sh}, A_{sh} patterns) was common in the lights-on period as lobsters made repeated explorations prior to shelter reoccupancy upon the light-cycle change (Fig. 5D).

Juvenile lobsters switched activities more frequently in the D phase than in the L phase. The rate of activity pattern change (changes per lobster per 30-min period) was comparable between the lights-off, midnight, and lights-on period (grand mean = 25.0 ± 20.06 (SD); $n = 60$), but was different between each of these periods and noon (3.9 ± 6.66 (SD); $n = 20$: Kruskal–Wallis test on ranked data, $P < 0.001$; non-parametric analog to Tukey test to establish differences between time periods, $P < 0.05$). General inactivity at noon was also reflected in frequency distributions of the duration of locomotor activity bouts (Fig. 7), the few A_{cs} and A_{op} bouts that were recorded at noon being <20 s in duration. Conversely, bouts of these activities up to 100 s in duration occurred at midnight, with longer bouts of A_{sh} (>140 s) being recorded. Bouts of A_{sh} occurring at noon were, with one exception, <60 s in duration.

Modification of Activity Pattern

Preliminary review of the video record for Experiment B revealed these lobsters, held for a long period in captivity, to be more active in the L phase than recently captured lobsters. In trials with individuals, L phase activity, primarily F_{sh}, was conducted from one of the two shelters provided and included sequestering of mussels for later consumption. High daytime activity levels in paired-lobster trials, whilst also feeding

TABLE 1. Time-budgeting within the L phase (12:00–14:00) and the D phase (19:00–21:00) by four juvenile lobsters monitored both singly and as two CL-matched pairs after each of three initial starvation periods (SP). The proportion of time spent in each of seven activity patterns by each lobster was coded (proportions in parentheses) prior to summation between lobsters monitored singly (Ind; $n = 4$) and as the dominant (Dom) or subdominant (Sub) member of a pair ($n = 2$ in each case): 0.5 (0–0.05), 1.0 (0.06–0.15), 2.0 (0.16–0.25), . . . , 9.5 (0.95–1.0). The maximum possible scores for an activity pattern were thus 38 (where $n = 4$) or 19 (where $n = 2$). Such scores would indicate that each lobster performed that activity pattern for >95% of the time period. Activity patterns W_{sh}, A_{sh}, and A_{cs} were combined for this analysis under *AS*; refer to the text for explanation of acronyms.

Initial SP (h)	Lobster status	L phase					D phase				
		AS	F_{sh}	I_{op}	A_{op}	F_{op}	*AS*	F_{sh}	I_{op}	A_{op}	F_{op}
24	Ind	25.0	14.5	—	—	1.0	11.5	3.5	—	21.0	3.0
	Dom	10.0	8.5	—	2.0	0.5	0.5	—	—	18.5	1.5
	Sub	2.0	—	5.5	4.0	8.0	0.5	—	5.0	15.0	—
48	Ind	9.5	28.5	—	—	—	13.5	9.5	—	16.0	1.0
	Dom	13.0	7.0	—	0.5	0.5	—	—	—	7.0	3.0
	Sub	17.5	2.0	—	0.5	0.5	0.5	—	1.0	18.0	0.5
72	Ind	5.0	33.5	—	—	—	15.0	11.0	0.5	10.0	4.5
	Dom	11.5	7.0	—	—	—	—	—	—	18.5	0.5
	Sub	12.0	6.0	—	1.0	1.0	—	—	3.0	17.0	0.5

related, were primarily a consequence of the dominance relationships established in those trials.

Time-budgets, constructed for individually monitored lobsters, revealed that an increased proportion of time in the L phase was allocated to foraging activity (primarily F_{sh}) after longer initial starvation periods (Table 1). F_{op} during the L phase was only observed in the 24-h treatment where two lobsters spent 1 and 5 min, respectively, engaged in this activity pattern. No individuals were observed I_{op} during the L phase. The rate of activity pattern change was low (1.83 ± 3.41 (SD) changes per lobster per 120-min period over all replicates), with some individuals remaining in one activity pattern throughout a recording segment.

In the D phase, the proportion of time engaged in foraging activity (F_{sh}, F_{op} patterns) also increased from the 24- to 72-h treatments; however, the total amount of time spent feeding was lower than in the L phase (Table 1). Lobsters were observed F_{op} during the D phase, but only one I_{op} bout was observed (Table 1). Individuals switched activities more frequently in the D phase (28.17 ± 16.70 (SD) changes per lobster per 120-min period; one-tailed Mann–Whitney test on ranked data, $P < 0.05$), spending approximately half of the time engaged in shelter-related activities (W_{sh}, A_{sh}, A_{cs}, or F_{sh} activity patterns) (Table 1).

The time-budgets of dominant and subdominant lobsters in paired-lobster trials were reciprocal. When the dominant lobster occupied shelter during the L phase and displayed minimal A_{op} activity, the subdominant lobster either occupied the other shelter or conducted F_{sh} and F_{op} activities (Table 1, 48- and 72-h treatments). A_{op} activity by the dominant lobster led to increased social interaction and, thus, a decrease in the proportion of time spent in shelter by the subdominant (Table 1, 24-h treatment) and concomitant increase in I_{op} and (forced) A_{op} activity patterns. Dominant lobsters were not observed I_{op} in any of the L phase or D phase recording segments subjected to time-budget analysis (Table 1).

Modification of activity pattern as a consequence of conspecific presence was most dramatic during the D phase. The proportion of time spent close to shelter ($AS = W_{sh} + A_{sh} + A_{cs}$; Table 1) was much reduced in paired-lobster trials by both dominant and subdominant lobsters and no F_{sh} activity was observed. A_{op} became the most significant component of the time-budget (Table 1); much of this time was spent in social interaction. Subdominance was evidenced by periods of I_{op} alternating with A_{op} upon approach by the dominant lobster.

A total of 167 social interactions were observed within the recorded segments analyzed for paired-lobster trials (24 h real time), distributed equally between the two CL-matched pairs (G test, $P > 0.05$). More interactions were initiated by dominant ($n = 134$) than by the subdominant lobsters ($n = 33$), but as these were also equally distributed between replicates (G test, both $P > 0.1$), data were combined for analysis. All three categories of social interaction, i.e. approaches in the open (8:99; *n*L:*n*D), shelter contests (11:37), and disturbance at feeding sites (2:10), occurred more frequently in the D phase (G tests, all $P < 0.05$). Approaches in the open were most common (107 approaches, 48 shelter contests, 12 feeding disturbances). The total number of social interactions was higher in the 24-h starvation period treatment ($n = 86$) than in the two other treatments (48 h: $n = 46$; 72 h: $n = 35$). The dominant lobsters won 133 of the interactions they initiated (99.25%) whereas the lobsters classed as subdominant were only successful once (3.03%) in encounters they initiated.

Foraging Behavior

Mussels were consumed primarily within shelter, foraging activities in the open being largely confined to prey acquisition. In the F_{sh} activity pattern, lobsters exited from shelter and moved directly onto the food patch. Following a short pause over the mussels, the lobster retired backwards toward shelter either carrying one mussel (held within one claw) or dragging a number of mussels (using both claws). The behavior was often repeated until as many as 10 mussels had been brought into the shelter. Subsequent appearance of shell debris at the shelter entrance indicated that feeding had commenced. Large mussels were sometimes redeposited outside the shelter (up to

TABLE 2. Mean ± SD number of mussels consumed over a 47-h period by juvenile lobsters monitored within 14 d of capture. The number of mussels presented is indicated in parentheses; n.p. = none presented.

	Mean number consumed within a size-class (mm SL)			
Lobster CL (mm)	5–10	10–15	15–20	20–25
20, 24, 26, 29	12.3 ± 2.8 (20)	6.5 ± 3.3 (10)	— (n.p.)	— (n.p.)
33, 38, 38	18.0 ± 0 (20)	18.0 ± 2.0 (20)	9.3 ± 0.6 (10)	— (n.p.)
42, 43, 46	— (n.p.)	16.3 ± 2.5 (20)	15.0 ± 5.0 (20)	7.3 ± 2.3 (10)

TABLE 3. Number of mussels consumed over a 22-h period by juvenile lobsters monitored singly and as CL-matched pairs after each of three initial starvation periods (SP). Ten mussels were presented in each size-class. The number consumed by each pair is followed (in parentheses) by the mean number consumed when the lobsters were monitored singly ($n = 2$). Also indicated is the amount of mussel flesh consumed by each pair (total individual consumption in parentheses) and the flesh DW estimated to be required for both lobsters to reach satiation after each initial starvation period.

		Number consumed within a size-class (mm SL)					Mussel flesh DW (g)	
Initial SP (h)	CL-matched group (mm)	5–10	10–15	15–20	20–25	25–30	Consumed	Required
24	34, 35,	1 (3.5)	3 (3)	3 (2.5)	3 (3)	0 (0)	0.58 (0.53)	0.32
	44, 44	10 (6)	10 (6)	7 (4)	9 (5)	8 (2.5)	1.65 (1.40)	0.69
48	34, 35,	7 (5.5)	7 (4)	2 (6)	4 (1)	0 (0)	0.39 (0.40)	0.34
	44, 44	6 (5.5)	7 (6)	6 (8)	8 (3)	4 (2.5)	1.10 (1.38)	0.75
72	34, 35,	7 (5.5)	4 (3.5)	5 (3.5)	5 (3)	0 (0.5)	0.48 (0.76)	0.50
	44, 44	9 (7.5)	9 (7.5)	10 (8)	10 (3.5)	9 (2.5)	1.72 (1.70)	1.10

0.15 m from the entrance) after being manipulated in the claws and rejected. Of 80 feeding bouts observed in Experiment A, more ($n = 58$) occurred in shelter than in the open (G test, $P < 0.001$). Feeding bouts in shelter lasted longer than those in the open (one-tailed normal approximation to the Mann–Whitney test using ranked data, $P < 0.01$). The mean durations of these feeding bouts, with 95% confidence intervals (derived from $\log_{10}(x + 1)$ transformed data), were $98.1 < 127.3 < 165.1$ s in the shelter and $21.9 < 36.4 < 59.9$ s in the open.

Lobsters typically consumed a greater DW of mussel flesh over the course of each experiment (22- or 47-h duration) than that predicted to be required after the initial starvation period (Tables 2, 3). In Experiment A, lobsters consumed mussels from different size-classes in proportion to mussel relative abundance (2:1 or 2:2:1 depending on prey size-class composition (Table 2); G tests (interactions), all $P > 0.1$; G tests (pooled data), all $P > 0.5$). A greater size range (and number) of mussels was presented to lobsters in Experiment B. In individual trials, lobsters primarily consumed mussels from the smaller size-classes (5–20 mm SL), but only fully depleted individual size-classes in three cases (out of 36 possible cases over all trials). The total number consumed from some small mussel size-classes by two lobsters monitored individually was greater than the number available to them when they were paired (mean n consumed by single lobsters >5.0; Table 3). This did not appear to be a problem in trials with the smaller CL-matched pair, as no mussel size-classes were fully depleted. Depletion of smaller mussel size-classes occurred in trials with the larger CL-matched pair; however, these lobsters supplemented their diet with large mussels (25–30 mm SL), and consequently maintained high flesh consumption levels (Table 3). Only one mussel from the largest size-class was consumed by the smaller CL-matched pair (over all trials; Table 3); these mussels were close to the critical size (Fig. 2), and may thus have been unavailable to these lobsters.

Discussion

Juvenile lobsters (20–46 mm CL) recently captured from the wild were primarily active nocturnally (Fig. 4), apportioning most of their time to activities conducted within or close to shelter (Fig. 5). Available field descriptions of juvenile lobster behavior corroborate these observations. Roach (1983) monitored survivorship, growth, and behavior of hatchery-reared stage IV lobsters released in an underwater enclosure in Lennox Passage, Nova Scotia. After 13 mo, a maximum size of 81 mm total length (approximately 28 mm CL; Wilder 1953, p. 393) had been attained among the surviving lobsters. No juveniles were observed actively moving over the bottom during diving surveys conducted in the enclosure. Some of the larger lobsters were recorded as being active at their shelter entrances, in what was termed "restless" behavior. Cooper and Uzmann (1980), diving in inshore lobster habitats off the northeastern United States, recorded juvenile lobsters 35–40 mm CL as "partially active at night," moving back and forth within their shelters and occasionally outside, activity patterns similar to the A_{sh} and A_{cs} patterns

documented in the present study. Ennis (1983) examined seasonal changes in depth distribution, nocturnal activity, and movement patterns in Bonavista Bay, Newfoundland, recording a male of 44 mm CL and a female of 46 mm CL as the smallest nocturnally active (errant) lobsters.

Nocturnal activity appeared cued to the experimental photoperiod. The abrupt changes in activity level (Fig. 4) and time-budget (Fig. 6) evident around each light-cycle change were likely a consequence of omitting crepuscular periods from the light regime. In shallow coastal waters, emergence from shelter of larger lobsters is apparently mediated by intermediate light intensities (Weiss 1970). Once established, nocturnal activity remained high until approximately 5 h after the onset of the D phase, decreasing thereafter in accordance with previous laboratory (Cobb 1977; Jacobson 1977; Wang 1982; Richards 1984) and field (Cooper and Uzmann 1980; Ennis 1983) observations on larger juveniles and adults.

The shelter-related foraging activities of early juvenile lobsters may represent a compromise between resource exploitation and predator avoidance. The nonselective acquisition of mussels in open areas of tanks observed in the present experiments contrasts markedly with the distinct prey acceptance–rejection sequences which occur as crabs forage on mussel patches (Elner and Hughes 1978; Hughes and Seed 1980). Crab foraging behavior may be appropriately modelled by reference to patch choice or optimal diet theory (Pyke 1984). The intimate association of lobsters with shelter, whether a central place (implying residency?) or available shelter (implying predator avoidance?), suggests that an alternative perspective is required, such as that offered by central-place foraging theory (Pyke 1984). Proximity and abundance of sessile epifauna (such as mussels) and encounter rates with mobile epifauna (such as crabs) may influence shelter-related foraging by juvenile lobsters, with food limitation being the proximate factor triggering the onset of wider ranging foraging behavior.

A similar ontogenetic pattern of resource use has recently been documented in spiny lobster, *Panulirus argus*, populations in the middle Florida Keys (Marx and Herrnkind 1985a). From settlement (6 mm CL) through to approximately 17 mm CL, *P. argus* remain within macroalgal (*Laurencia* spp.) clumps which provide both refuge from predation and support an abundant, diverse prey fauna. Subsequently, juvenile lobsters move onto the substrate, finding refuge in crevices and foraging nocturnally in a narrow zone around these (Andree 1981). Eventually, they assume the crepuscular–nocturnal foraging behaviors and den residency patterns of adult spiny lobsters (Lipcius and Herrnkind 1985). Laboratory studies (Marx and Herrnkind 1985b) indicate that trophic pressures, rather than physical sheltering factors, regulate habitation patterns of algal-dwelling *P. argus*. Predation risk was cited (Andree 1981; Marx and Herrnkind 1985a) as a potentially important selective factor underlying the association of early benthic *P. argus* with *Laurencia* and other similar vegetation.

In studies on interspecific interactions between crabs and lobsters (Krekorian et al. 1974; Wang 1982; Richards 1984), it has been noted that crabs, once they leave their shelters at dusk, tend to remain out of shelter until they take up residency once again at dawn. Lobsters, however, typically alternate between periods of shelter occupancy and general activity. Ennis (1983) observed lobsters in the wild to be in shelters during the first few hours of darkness feeding on animals that they probably had left their shelters to find. These observations support the hypothesis that lobsters are strongly adapted (and tied) to shelter use (Cobb et al. 1986) and, additionally, may be explicable in terms of central-place foraging behavior.

When individual hunger level was increased or when intraspecific interaction was imposed (Experiment B), juvenile lobsters modified their diel activity pattern and time-budgets in manners which (intuitively) would increase predation risk. In individual-lobster trials, foraging in the L phase increased as initial starvation period was raised; however, lobsters continued to consume mussels primarily within shelter. In paired-lobster trials, subdominant lobsters foraged during the L phase, exploiting periods when the dominant lobster was inactive, but tended to forage in the open. Due to the monopolization of shelter by the dominant lobster, a common occurrence among communally held lobsters (Jacobson 1977; Wang 1982), subdominant lobsters also exhibited periods of inactivity in the open. This behavior pattern, rarely seen in individual-lobster trials, was never expressed by dominant lobsters (Table 1).

As observed in other studies of aggressive interaction between lobsters (Scrivener 1971; Stein et al. 1975; Jacobson 1977; Wang 1982), the majority of social interactions, once dominance was established, took the form of simple approach–avoid sequences, entailing no physical contact. Active competition for shelter followed the pattern described by O'Neill and Cobb (1979) and Atema and Cobb (1980), including ritualized shelter eviction behavior (fig. 1 in O'Neill and Cobb 1979). In all cases ($n = 12$) where interactions occurred at feeding sites, the dominant lobster was successful, indicating that dominance may secure priority access to food resources (as speculated by Cobb et al. 1982). Although dominant lobsters in CL-matched pairs exhibited greater feeding rates, based on the video record and the distribution of mussel shell debris (P. Lawton, pers. obs.), subdominant lobsters continued to forage, primarily when the dominant lobster was inactive. Previous studies (referenced by Cobb et al. 1982) have indicated reduced food intake to be the primary consequence of subdominance status among decapod crustaceans maintained under communal laboratory conditions. In field situations, where predation risk may be significant (Cooper and Uzmann 1980), the activity pattern changes accompanying subdominance may be of greater importance than reduced food intake.

The extent to which agonistic interactions are important in natural populations has been the focus of considerable debate. A review by Atema and Cobb (1980) concluded that whereas aggression in lobsters may serve to secure adequate shelter, formal dominance orders or territories probably do not exist, being ineffective in lobster populations which are characteristically semi-nomadic (Stewart 1972; Ennis 1983). Atema and Cobb's (1980) conclusion apparently derives from considerations of shelter and food utilization by late juvenile and adult lobsters. Given the presently defined ontogenetic pattern of resource utilization by *H. americanus*, it is plausible that dominance and territoriality may exist early in ontogeny. Prolonged occupation and defense of a shelter adjacent to an abundant food resource, such as a mussel patch, may be an effective strategy for early juveniles to follow if the lobster population is spatially segregated with respect to size (e.g. among early juveniles occupying shallow inshore nursery areas). Similar arguments were developed by Marx and Herrnkind (1985b) for *P. argus* occupying shallow habitats in the Florida Keys.

An understanding of the diel activity and movement patterns of juvenile lobsters appears crucial for the design and interpretation of prerecruit abundance surveys. Diel activity as determined in this study, using recently captured juvenile lobsters, probably resembles that in the natural population during summer months. No measurements were taken, however, of the distance moved by juvenile lobsters during periods of general locomotor activity. These would have been unrepresentative of movement in natural habitats where near-bed tidal currents, wave-induced currents, and substrate topography influence lobster movement (Howard and Nunny 1983). Although primarily reporting on development of sampling and tagging methodologies for small juvenile lobsters, Bernstein and Campbell (1983) presented data on the recapture of tagged lobsters from 50-m^2 quadrats (5 × 10 m) which suggest that juveniles may remain in such restricted areas for periods up to 7 wk. More detailed field observations should be undertaken on the shelter residency patterns of juvenile lobsters, perhaps utilizing underwater video techniques and incorporating assessments of food availability in the immediate shelter vicinity. If foraging movements of juvenile lobsters prove to be restricted, then it is apparent that recruitment monitoring via juvenile-directed trapping programs would require an intensive fishing effort, as such traps would have limited fishing areas. Paradoxically, rather than prefacing enhanced recruitment to the exploited stock, high capture rates in juvenile-directed traps may simply reflect shelter and/or food limitation in nursery areas and, potentially, high natural mortality rates among prematurely wide-ranging juvenile lobsters.

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Role of Environment and Stock on the Elemental Composition of Sockeye Salmon (*Oncorhynchus nerka*) Vertebrae

S. Behrens Yamada, T. J. Mulligan, and D. Fournier

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

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Individual spawning populations, or stocks, of salmon differ in the elemental composition of their scales and bone. These stock-specific differences have a pronounced environmental component, since elevated concentrations of certain elements in fish tissue can be induced by feeding hatchery fish food enriched in these elements. This study tests the hypothesis that both genetic and environmental factors contribute to the stock-specific elemental composition of salmon vertebrae. Four stocks of sockeye salmon (*Oncorhynchus nerka*) were raised under identical conditions and each stock was subjected to four environmental treatments. The elemental composition of the vertebrae were analysed using X-ray spectroscopy; nine elements were measured. The resulting elemental composition data were analysed by multivariate analysis of variance. Evidence is presented for both a stock and a treatment factor contributing to the vertebral composition.

Les populations de frai, ou stocks, des saumons diffèrent de par la composition chimique des écailles et des os des poissons. L'environnement a un effet prononcé sur ces différences spécifiques entre les stocks car il est possible d'obtenir en pisciculture des concentrations élevées de certains éléments dans les tissus des poissons en leur donnant une nourriture enrichie de ces mêmes éléments. Les auteurs ont testé l'hypothèse voulant que des facteurs génétiques et environnementaux influent sur la composition chimique des vertèbres des saumons de certains stocks. Des saumons nerka (*Oncorhynchus nerka*) de quatre stocks ont été élevés dans des conditions identiques et chaque stock a été soumis à quatre régimes environnementaux. La composition chimique des vertèbres a été déterminée par spectroscopie aux rayons X et neuf éléments ont été dosés. Les données sur la composition en éléments ont ensuite fait l'objet d'une analyse de variance à variables multiples. Les auteurs présentent des résultats indiquant que la composition des vertèbres est due à un facteur inhérent au stock et à un facteur lié au traitement.

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The determination of the catch attributed to a Pacific salmon stock is frequently complicated by fisheries operating on stock mixtures of unknown composition. It is desirable to identify these stocks for management of the fisheries or for evaluation of their biological productivity. Various techniques including mark and recapture, distinct morphological, biochemical and scale patterns, and characteristic parasites have been employed in identifying the origin of salmon.

Recently the feasibility of using the elemental chemical composition of salmon tissue as a stock discrimination tool has been studied. Bagenal et al. (1973) were able to distinguish resident and sea-run populations of brown trout (*Salmo trutta*) based on the strontium (Sr) concentration in their scales. Mulligan et al. (1983) found that sockeye salmon (*Oncorhynchus nerka*) from five Alaska and three Vancouver Island stocks could be classified to their stock of origin with an accuracy of 80–95% on the basis of the elemental composition of vertebrae. The freshwater portions of the scales from the same Vancouver Island stocks were examined using scanning electron microscopy combined with energy dispersive X-ray analysis. This technique proved to be less accurate, yielding a correct stock classification of 63% (Lapi and Mulligan 1981).

Since salmon spend part of their life cycle in their home stream, it is conceivable that the observed stock-specific elements have a strong environmental component. Abdullah et al. (1976) found a strong correlation between the Mn and Zn concentration of salmonid scales and that of their freshwater environment. Other investigators demonstrated that the addition of such elements as Eu, Sr, and Mn to the food and/or water of growing salmon resulted in elevated concentrations of these elements in their scales and bones (Yonemori et al. 1972; Behrens Yamada et al. 1979; Behrens Yamada and Mulligan 1982, and unpubl. data).

Although it is known that environmental factors can play an important role in the elemental composition of salmon bones and scales, it is not known if genetic factors are also important. Our study tests the hypothesis that a genetic component also contributes to the characteristic elemental composition of salmon vertebrae. Differences among stocks which are not due to the environment are referred to as genetic factors. To test this hypothesis, gametes of fish from geographically separated populations were collected and progeny were reared in a hatchery under identical environmental conditions. Results of the subsequent elemental analysis of vertebrae from these fish were subjected to multivariate analysis of variance

TABLE 1. Elemental composition of the diets. Only those elements which differed substantially among the diets are shown in arbitrary relative concentration units.

Diet	P	Sr	Mn
Control	27.2	11.4	49.5
Sr food	64.3	21800	51.0
Mn food	21.9	10.4	26000

(MANOVA) to test for the influence of both an environmental and a genetic factor.

The purpose of our study was twofold. First, we wanted to test the hypothesis that genetic factors can influence the elemental composition of fish tissue. This would help to explain the mechanisms behind observed differences in elemental composition of wild stocks of salmon. Second, we wanted to continue with our work on marking fish by inducing "elemental tags," by testing if the stock factor influenced the uptake of elements from the treated diets or water. If stock did play a significant role in this uptake, then marking fish with this technique might require studying the specific stocks involved.

Experimental Methods

Experimental Design

Eggs and sperm from four geographically distinct sockeye salmon populations were collected in the fall of 1976. These populations, geographical coordinates, and sampling dates were as follows: Fulton River (54°46′N, 126°13′W), September 21; Genesee Creek (51°44′N, 126°40′W), October 21; Kennedy Lake (49°3′N, 125°30′W), November 8; Cultus Lake (49°4′N, 121°58′W), November 23. Eggs from at least 20 females and sperm from at least 20 males were collected from each stock and kept cold during transport to the Rosewall Creek Hatchery. Eggs were fertilized at the hatchery and reared according to standard hatchery practice.

Since spawning was not simultaneous in these stocks, rearing temperatures were manipulated such that by the end of May 1977, fish from all four stocks were roughly of the same size and at the same stage of development. Some of the Fulton River fish were also reared at ambient temperatures to determine whether temperature manipulation affected their elemental uptake.

Each stock was subdivided among four experimental treatments: control, Sr-in-food, Mn-in-food, and Sr-in-water. Thus there were 16 stock–treatment cells in a factorial experimental design. Two additional cells were composed of large (ambient temperature) Fulton River fish for control and Sr-in-food treatments. Within each cell, fish were assigned to five rearing tanks (35 L; 2 L/min flow rate). The locations of the 90 rearing tanks were randomly assigned. Either 100 large Fulton River fish reared at ambient temperature or 200 smaller temperature-manipulated fish were introduced into each tank. Experimental treatment commenced June 20 and terminated August 19.

Preparation of Experimental Diets

Experimental diets were prepared by adding strontium chloride or manganese chloride to the standard hatchery diet. To achieve an additional Sr or Mn concentration of 10 mg/g in the diet, 91.5 g of $SrCl_2 \cdot 6H_2O$ or 108 g of $MnCl_2 \cdot 4H_2O$ was dissolved in 1 L of distilled water and added to 3 kg of starter mash. The mixture was blended for 6 min, frozen, chopped, and forced through a commercial meat grinder having a die with 1-mm-diameter holes. The "spaghetti-like" strings of food leaving the meat grinder were frozen, crumbled by hand, sieved, and refrozen. Food particles were subsequently dried for 3 h in a fume hood at room temperature until the finished product resembled the commercially available 0.119-cm food pellets in size, color, and water content. Food for the control treatments was prepared in a similar fashion. All prepared diets were frozen until used during the treatment period. X-ray spectroscopic elemental analysis of the various diets demonstrated that only three elements varied substantially among diets (Table 1). The higher P concentration in the Sr-enriched diet was unintentional with unknown cause.

TABLE 2. Mean fish weights (g) on September 26.

Stock	Control food	Sr food	Mn food	Sd water
Fulton	6.1	6.4	6.2	6.1
Genesee	6.2	6.9	6.1	6.8
Kennedy	5.6	5.7	5.1	5.3
Cultus	6.6	6.7	6.2	6.6

Addition of Sr to the Water

A stock solution of strontium chloride (6.1 g $SrCl_2 \cdot 6H_2O$/L) was added to the water supply of the Sr-in-water treatment tanks using a multichannel peristaltic metering pump. The flow rates of the water supply hoses were adjusted to 2 L/min ± 5% and those of the metering pump to 1 mL/min ± 5%. Thus the Sr concentration of the water in the tanks receiving the stock solution was 1 μg/g greater than that in the rest of the treatments.

Sample Preparation

Fish were sampled biweekly throughout the treatment period and monthly thereafter. Specimen pellets of whole individual fish and vertebrae samples pooled from fish from the same tank were prepared by freeze drying the material for 2 d followed by grinding it for 3 min in a mixer mill. One tenth of a gram of each powdered sample was pressed into a pellet 1.27 cm in diameter using a die and hydraulic press with an applied load of 2200 kg. Since whole fish samples showed great variation in elemental composition within each cell of the experimental design, our analysis used vertebrae samples only. Elemental analysis was performed on vertebrae samples taken on November 3, 1977, 75 d after cessation of treatment.

Elemental Analysis

The sample pellets were analysed for their elemental concentrations using an X-ray fluorescence spectrometer (Nuclear Semiconductor, Spectrace 440). Elements with atomic number >10 and <39 are efficiently detected by this method; heavier elements are less efficiently detected (Woldseth 1973). Quantitative determination of the elements in each sample was accomplished by the method of Giaque et al. (1973). Further details of the spectroscopic analysis can be found in Mulligan et al. (1983).

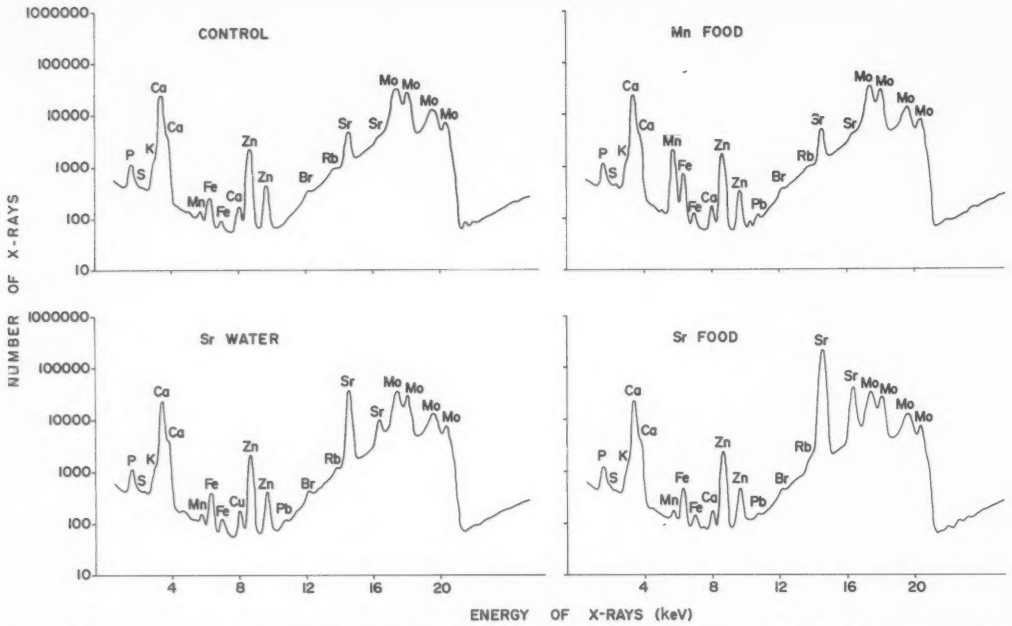

FIG. 1. Sample X-ray spectra for representative fish vertebrae from each of the experimental treatments. The elements responsible for the structure in the spectra are identified by their chemical notation.

TABLE 3. Number of observations per cell of the experimental design.

Stock	Treatment			
	Control food	Sr food	Mn food	Sr water
Fulton	13	12	12	13
Genesee	12	13	10	13
Kennedy	12	12	11	8
Cultus	12	12	9	12

While the primary focus of our study was the elemental composition of bones, we also learned about the effects of stock and treatment on growth through examination of the fish weight data. A two-way ANOVA was performed with fish weight as the dependent variable for each of the following dates: July 5, August 4, and September 26 (Table 2).

Data Analysis

Some of the problems encountered in the X-ray spectra were due to interelemental interferences. More abundant elements such as Sr cause problems when measuring less abundant elements such as Rb. This interference made the accurate assessment of Rb very difficult; therefore, Rb was not included in the data analysis. Using the same reasoning, we also eliminated Pb and Fe. We were left with the concentrations of the following nine elements: P, S, K, Ca, Mn, Cu, Zn, Br, and Sr (Fig. 1).

This experiment represents a classical two-way factorial design (e.g. Snedecor and Cochran 1980). The two factors are treatment (diet or water) and stock and each has four possible values (Table 3). A series of nine preliminary 2 × 2 ANOVA's was performed on the Fulton low temp, Fulton ambient temp, control food, and Sr food cells (one ANOVA for each of the nine elements listed above). The results of these analyses demonstrated no stock difference between the two Fulton groups of fish. We therefore analysed the data as a 4 × 4 two-way factorial design with unequal numbers of observations per cell. MANOVA was used to include the effects of interelemental correlations. The MANOVA model tested for treatment, stock, and treatment–stock interaction factors using the following set of equations for our model:

$$(1)\quad y_{ijkl} = \mu_k + \alpha_{ik} + \beta_{jk} + \gamma_{ijk} + \epsilon_{ijkl}$$

$$(2)\quad \sum_i \alpha_{ik} = 0 \text{ for each value of } k$$

$$(3)\quad \sum_j \beta_{jk} = 0 \text{ for each value of } k$$

$$(4)\quad \sum_i \sum_j \gamma_{ijk} = 0 \text{ for each value of } k$$

where y represents the measured elemental concentration, μ represents the overall mean, α represents the stock factor, β represents the treatment factor, γ represents the stock–treatment interaction factor, ϵ represents the error covariance matrix distributed $N(O,\Sigma)$, i indexes stock, j indexes treatment, k indexes element, and l indexes observation number. These equations represent the standard MANOVA model which is discussed in many texts (e.g. Bock 1975, chap. 5).

The original elemental concentrations from our measurements did not satisfy the model assumptions of normality and homogeneity of variance. The MANOVA was performed on ln-transformed (log base e) values of the elemental concentrations. Transformation resulted in normal distributions

TABLE 4. Means and standard deviations for each cell and each element used in the MANOVA. The means are for the ln-transformed elemental concentrations (μg/g). The columns and rows under each element correspond to the location of the cells in the experimental design of Table 3.

Element	Cell means (SD)			
P	12.36 (0.20)	12.41 (0.12)	12.28 (0.19)	12.30 (0.16)
	12.32 (0.19)	12.47 (0.10)	12.29 (0.15)	12.37 (0.06)
	12.30 (0.13)	12.42 (0.06)	12.31 (0.11)	12.28 (0.19)
	12.38 (0.09)	12.45 (0.08)	12.28 (0.15)	12.38 (0.04)
S	9.15 (0.18)	8.90 (0.26)	9.05 (0.17)	9.05 (0.18)
	9.00 (0.19)	8.98 (0.24)	9.13 (0.15)	9.03 (0.11)
	9.19 (0.23)	8.99 (0.20)	9.23 (0.15)	9.06 (0.17)
	9.02 (0.23)	9.06 (0.28)	9.13 (0.12)	9.04 (0.16)
K	9.51 (0.29)	9.43 (0.30)	9.52 (0.27)	9.34 (0.16)
	9.46 (0.27)	9.34 (0.16)	9.23 (0.21)	9.34 (0.32)
	9.36 (0.11)	9.20 (0.22)	9.33 (0.30)	9.38 (0.24)
	9.46 (0.30)	9.18 (0.29)	9.38 (0.32)	9.32 (0.29)
Ca	11.96 (0.10)	11.87 (0.12)	11.91 (0.07)	11.87 (0.05)
	11.91 (0.06)	11.90 (0.10)	11.89 (0.04)	11.92 (0.05)
	11.87 (0.05)	11.84 (0.07)	11.89 (0.06)	11.88 (0.09)
	11.95 (0.07)	11.85 (0.10)	11.88 (0.04)	11.93 (0.05)
Mn	3.60 (0.17)	3.62 (0.20)	6.97 (0.30)	3.45 (0.17)
	3.49 (0.22)	3.60 (0.20)	7.06 (0.24)	3.60 (0.15)
	3.58 (0.15)	3.62 (0.14)	7.07 (0.33)	3.62 (0.18)
	3.28 (0.10)	3.48 (0.29)	7.20 (0.30)	3.36 (0.19)
Cu	2.81 (0.30)	2.84 (0.33)	2.61 (0.29)	2.65 (0.16)
	2.64 (0.46)	2.75 (0.28)	2.79 (0.25)	2.58 (0.27)
	2.86 (0.30)	2.74 (0.22)	3.09 (0.40)	2.75 (0.35)
	2.63 (0.33)	2.91 (0.19)	2.62 (0.28)	2.70 (0.35)
Zn	5.57 (0.14)	5.45 (0.18)	5.36 (0.10)	5.50 (0.14)
	5.13 (0.14)	5.15 (0.16)	5.01 (0.10)	5.13 (0.17)
	5.26 (0.17)	5.20 (0.20)	5.19 (0.15)	5.32 (0.23)
	5.41 (0.12)	5.39 (0.19)	5.14 (0.06)	5.37 (0.12)
Br	1.59 (0.20)	1.52 (0.21)	1.58 (0.18)	1.55 (0.20)
	1.53 (0.28)	1.42 (0.18)	1.31 (0.10)	1.43 (0.27)
	1.55 (0.19)	1.35 (0.16)	1.47 (0.19)	1.55 (0.24)
	1.40 (0.22)	1.32 (0.24)	1.38 (0.21)	1.43 (0.27)
Sr	5.57 (0.13)	9.03 (0.22)	5.63 (0.18)	7.26 (0.17)
	5.57 (0.11)	9.02 (0.24)	5.49 (0.13)	7.33 (0.21)
	5.56 (0.15)	9.08 (0.26)	5.52 (0.15)	7.28 (0.22)
	5.54 (0.15)	9.08 (0.26)	5.52 (0.15)	7.26 (0.22)

within each cell and reasonably homogeneous standard deviations (see Table 4).

We initially used a MANOVA procedure which formed part of a commercial statistical analysis system (OSIRIS).[1] Although we had no problems obtaining results, we did have problems interpreting these results. The difficulty arose because the commercial system did not specify the hypothesis tests it performed. With unbalanced data, as in our case, the solution of the model equations becomes more difficult and a series of hypothesis tests is required to choose the optimum model (Searle 1971, chap. 7). We used our own version of a general linear model parameter estimation algorithm (Lawson and Hanson 1974) coupled with a series of prototype subroutines to obtain the model parameter estimates and perform the associated significance tests. Our confidence in our results was reinforced by noting that three of our hypothesis tests appear to be identical to the ones used by the commercial analysis package as the only tests it performed.

[1]Other commercial software packages such as SAS and SYSTAT also contain MANOVA algorithms which will accomodate unbalanced data.

TABLE 5. Significance tests for MANOVA models. The degrees of freedom for the denominator in Rao's F ratio have been rounded to the nearest integer.

Significance test		Rao's F	$F\alpha=0.01$
Number	Model[a]	(ν, δ)	(ν, δ)
1	$F(\alpha\|\mu)$	7.43 (27, 509)	2.12 (27, 500)
2	$F(\beta\|\mu)$	286 (27, 509)	2.12 (27, 500)
3	$F(\alpha, \beta\|\mu)$	84.3 (54, 877)	1.64 (55, ∞)
4	$F(\alpha, \beta, \gamma\|\mu)$	24.7 (135, 1276)	1.37 (125, ∞)
5	$F(\beta\|\mu, \alpha)$	302 (27, 500)	2.12 (27, 500)
6	$F(\alpha\|\mu, \beta)$	9.10 (27, 500)	2.12 (27, 500)
7	$F(\gamma\|\mu, \alpha, \beta)$	1.47 (81, 1056)	1.49 (80, ∞)

[a]Notation such as $F(\alpha|\beta, \mu)$ is taken from Searle (1971) and describes the F-test for fitting the α factor aftering having fit the β and μ factors.

Our solution to the MANOVA is summarized as follows. In general matrix notation, equations (1) to (4) can be written as

$$\boldsymbol{O} = \phi \boldsymbol{P} + \Sigma$$

where $\boldsymbol{O}$ is the observation matrix, ϕ is the design matrix, $\boldsymbol{P}$ is the parameter vector, and Σ is the error matrix. Minimizing the quantity

$$\| \boldsymbol{O} - \phi \boldsymbol{P} \|^2$$

with respect to $\boldsymbol{P}$ provides the parameter estimates, a residual vector, and an estimate of the parameter covariance matrix (dispersion matrix). The residual vector can be used to examine the fit of various models, while the significance tests are made using Rao's F statistic (Rao 1973, p. 556). This statistic can be constructed from the ratio of the product of eigenvalues of the dispersion matrix, and it tests for the significance of improved fit between a model with greater and a model with lesser number of parameters.

Results

Some dramatic differences are seen in the elemental composition of the fish vertebrae when the data are averaged by treatment or by stock (Fig. 2). The Sr concentration was ~33 times greater in fish fed the Sr-enriched diet and ~5.6 times greater in fish reared in Sr-enriched water compared with the control fish. The four stocks did not differ significantly in their Sr concentrations. The Mn concentration was ~33 times greater in Mn treated than in control fish, with no significant

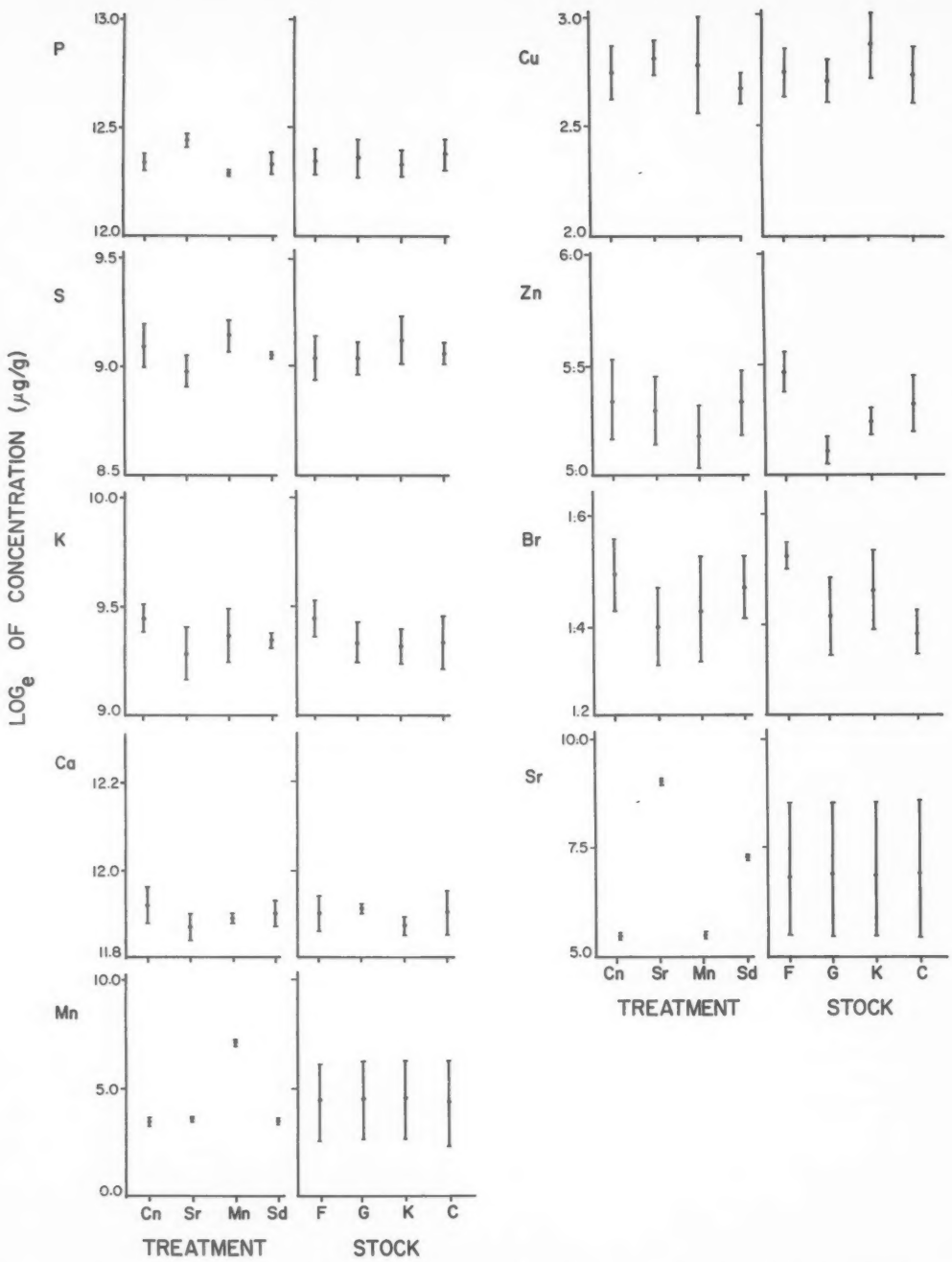

FIG. 2. A pair of plots for each element used in the MANOVA demonstrates the variation observed when averaging over treatment or over stock. Each pair of plots is identified by its chemical notation in the upper left-hand corner. The error bars represent ± 1 SD.Cn, control; Sr, Sr-enriched diet; Mn, Mn-enriched diet; Sd, Sr added to the water; F, Fulton River; G, Genesee Creek; K, Kennedy Lake; C, Cultus Lake.

differences among stocks. The Sr treatments did not affect the Mn concentration of the samples and vice versa. The Zn concentration was statistically different among the four stocks of our experiment. All other elements showed no statistically significant stock effect.

The above summary was substantiated by the MANOVA. The ln-transformed elemental concentrations were fit with several different forms of the MANOVA model and a series of

TABLE 6. Norms of the residual vector listed by model and element.

Element	No parameters	μ	$\mu + \alpha$	$\mu + \beta$	$\mu + \alpha + \beta$	$\mu + \alpha + \beta + \gamma$
P	168.5	1.950	1.923	1.799	1.782	1.753
S	123.6	2.805	2.764	2.698	2.659	2.571
K	127.7	3.652	3.574	3.559	3.481	3.385
Ca	162.2	1.057	1.033	1.014	0.995	0.953
Mn	62.47	20.39	20.33	3.034	2.970	2.807
Cu	37.71	4.323	4.228	4.255	4.169	3.951
Zn	72.22	2.895	2.215	2.769	2.046	1.988
Br	20.17	3.052	2.913	2.994	2.853	2.794
Sr	96.16	19.92	19.91	2.506	2.501	2.448

TABLE 7. Significance tests for ANOVA of fish weight for the September 26 sample.

Source	df	Sum of squares	Mean square	*F* value	Prob.
Model	15	281.98	18.799	8.27	0.0001
Treatment	3	37.358	12.453	5.48	0.0010
Stock	3	218.78	72.926	32.1	0.0001
Treatment × stock	9	25.841	2.8713	1.26	0.2525
Error	1075	2442.6	2.2722		

significance tests was performed (Table 5). These tests assumed a fixed effects model, while allowing correlation between all possible pairs of elemental concentrations within each sample. Significance tests 1–4 show that the full model and the partial models all provide an improved description of the data as compared with all cells in the design having a common mean, a model with only the μ term. To guard against Type I statistical errors and to provide evidence for the optimum model, further *F*-tests are necessary (Searle 1971). The further tests 5 and 6 show that both treatment (β) and stock (α) effects give improved fit when compared with a one-factor model. In all the above tests, those testing treatment effects (particularly 2 and 5) are dramatic, while the stock effect tests (1 and 6) are much less pronounced, yet still highly significant. Test 7 indicates a marginal stock–treatment interaction.

A much less pronounced, though still significant, treatment effect was observed for P. The Sr-enriched diet also had higher P content than the other diets (Table 1). Although this P manipulation was unintentional, it is the likely mechanism behind the treatment effect for this element.

The residual norms (mean magnitude of residuals) of Table 6 reinforce the above interpretation. Residuals for Sr and Mn were dramatically reduced when the treatment factor was included in the model. Only Zn showed a significant stock effect for the stocks examined in this experiment.

The results of the fish weight analyses (Table 7) indicated that the treatment and stock factors were significant. As time passed during the treatment period, treatment effects became more pronounced. No significant stock–treatment interaction was observed. The fish weights for the September 26 sample (Table 2) are typical of all the weight data. The Mn treatment is seen to have a deleterious effect on the fish, causing them to grow less rapidly than fish in the other treatments. Fish from the Kennedy Lake stock consistently weighed less than the average, while Cultus Lake fish tended to weigh more. After cessation of the treatments the Mn-treated fish grew as quickly as the other fish.

Conclusions

The environmental factors (diet and water treatments) produced much more pronounced effects in our study than did the stock factor. However, the stock factor was significantly different for the concentration of Zn in the fish vertebrae. No differences among stocks for the treatment elements, Sr and Mn, were observed. The references we cited in the introduction have demonstrated that dramatic changes in elemental composition are possible as the result of diet or water treatment. This study agrees with past findings. We are unaware of any previous work which looked for a genetic factor as a source of variation in elemental composition. We do not claim that our results are typical for all possible salmon stocks; rather, we claim to have demonstrated that the genetic character of a salmon can influence the elemental composition of its vertebrae.

These experimental fish probably differ considerably from naturally raised fish with respect to the extreme dominance of environmental (treatment) effects as compared with genetic (stock) effects. In nature it would be rare indeed to find food sources as radically different as those we artificially created. Our Sr diet had ~2000 times the Sr concentration of the control diet, while the Mn diet had ~500 times the Mn concentration of the control. We believe that the elemental composition of fish tissues can be affected by both environmental and genetic factors and shall not speculate about their relative importance under natural conditions.

The analyses of fish weights demonstrate pronounced stock and treatment effects. While differences among stocks are well known, the observed treatment effects should caution prospective proponents of dietary manipulation to study the possible adverse effects before using this technique to mark large numbers of artificially raised fish. The influences of various elements are diverse and not well studied.

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Separable Virtual Population Analysis of Pacific Salmon with Application to Marked Chinook Salmon, *Oncorhynchus tshawytscha*, from California's Central Valley

Robert G. Kope

Department of Wildlife and Fisheries Biology, University of California, Davis, CA 95616, USA

Kope, R. G. 1987. Separable virtual population analysis of Pacific salmon with application to marked chinook salmon, *Oncorhynchus tshawytscha*, from California's Central Valley. Can. J. Fish. Aquat. Sci. 44: 1213–1220.

A separable virtual population analysis model is developed for Pacific salmon which utilizes aged catch and spawning escapement data. This model is applied to marked chinook salmon, *Oncorhynchus tshawytscha*, from California's Central Valley hatcheries using weighted least squares criteria for goodness of fit. Structural inadequacies of the model apparently produce discrepancies between predicted values and observed data that are of about the same magnitude as the observational errors in estimating the recoveries of marked fish. Some of the inadequacy of the model may be due to environmentally induced variability in population parameters, but for the marked fish used in this analysis, some of the variability is probably due to year-to-year variability in hatchery practices. From this analysis it appears that although nominal fishing effort has been relatively stable or even declining in recent years, fishing mortality has been increasing with the exception of 1983 and 1984.

L'auteur met au point un modèle séparable d'analyse de population virtuelle des saumons du Pacifique à l'aide de données sur l'échappée et les captures d'âge déterminé. En se servant de la méthode des moindres carrés pondérés pour la validité de l'ajustement, il applique ce modèle à des saumons quinnat, *Oncorhynchus tshawytsha*, marqués provenant de piscifactures de la vallée centrale de la Californie. Des défauts structuraux du modèle produisent selon toute apparence des écarts entre les valeurs prédites et les valeurs observées qui sont presque de la même grandeur que les erreurs d'observations pour ce qui est de l'estimation de la récupération des poissons marqués. Certains des défauts du modèle peuvent être le résultat d'une variabilité des paramètres démécologiques provoquée par l'environnement quoique dans le cas des poissons marqués utilisés dans la présente analyse, une certaine partie de la variabilité vienne probablement de variations des méthodes d'élevage d'une année à l'autre. D'après cette analyse, il semble que la mortalité par pêche soit à la hausse sauf en 1983 et 1984, malgré que l'effort de pêche nominal ait été relativement stable ou même à la baisse au cours des dernières années.

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Techniques of cohort analysis or virtual population analysis are widely used to estimate historical population abundances and exploitation patterns in fisheries. These techniques are based on the catch equations of Baranov (1918) or on discrete time harvest–survival equations. These models generally assume a closed population in which all losses from a cohort can be accounted for by a constant natural mortality rate and constant annual fishing mortality rates.

The parameters of these models are fit to catch-at-age data either from a single individual cohort or from several cohorts at once. In the single cohort approach, when there are m age-classes in the cohort, values of $m + 2$ parameters (recruitment of the youngest age-class, the natural mortality rate, and m fishing mortality rates) are required. With only m data points the problem is clearly underdetermined, so it is necessary to specify the values of two parameters (usually natural mortality rate and fishing mortality rate for the oldest age-class) before a unique solution can be obtained. Values of the fishing mortality rates and cohort abundance are then estimated sequentially for earlier years either by solving approximations to the catch equations (Fry 1949; Jones 1964; Pope 1972) or by iterative solutions with a computer (Sims 1982; Huang and Walters 1983). Natural mortality rate must be independently estimated, and it has been shown that estimates of recruitment and of fishing mortality in earlier years are relatively insensitive to the value of fishing mortality chosen for the oldest age-class (Ulltang 1977; Pope and Shepherd 1982) .

Limitations of individual cohort techniques include the following: (1) estimation errors that arise in generating the catch-at-age data are ignored; (2) fishing mortality cannot be directly separated into annual fishing effort and the vulnerability of different age-classes to the fishery; (3) current abundance can be estimated only by using additional data and assumptions; and (4) the number of parameters estimated is equal to the number of data points, so solutions precisely match the catch record. As a consequence of the last limitation, there is no measure of variability and no way to establish confidence limits for the estimates of fishing mortality and abundance.

In practice, these limitations are dealt with or justified in a variety of ways. The errors in estimating catch-at-age are relatively small in comparison with other types of fishery data (e.g. abundance estimates from catch-effort data). Fishing effort and vulnerability at age may be estimated from the matrix of fishing mortalities resulting from the analysis of several cohorts. Pa-

rameters may be estimated for cohorts that are currently in the fishery by using catch-effort data to estimate abundance in the most recent year, and the number of parameters may be reduced by additional assumptions about the relationship between vulnerability and age.

Most of the limitations of the individual cohort techniques can be dealt with collectively by using multiple cohort techniques, here called separable virtual population analysis because the fishing mortality rates are separated into annual fishing effort and vulnerability at age. Instead of using a single vector of catch-at-age data for one cohort, multiple cohort techniques use a matrix of catch-at-age data for fish of all ages in all years. All data containing information about a parameter are used in the estimation of that parameter. By decomposing fishing mortality rates into annual fishing effort and the vulnerability of each age-class, the number of parameters is reduced so that there are more data points than parameters. If there are catch data for n years and for m age-classes, then the nm estimates of fishing mortality rates become n estimates of effort and m estimates of vulnerability at age. The problem then becomes one of finding the values of recruitment, effort, and vulnerability that will best fit the catch-at-age matrix.

There are two basic sources of error or discrepancy between the catch-at-age matrix generated by the model and the matrix of observed catch-at-age data: (1) violation of the assumptions of the model and (2) errors in the "data" (i.e. estimates of catch-at-age) to which the model is fit. In the first category, the natural mortality rate may vary with age and with time, the availability of fish to the fishery may vary from year to year, the force of fishing may not be proportional to fishing effort, and the population may not be closed. Even if the assumptions of the model are not violated with respect to population parameters, there will be some variability in catches, since fishing is a stochastic process. However, this variability will be relatively low when numbers are large. The second source of error results from estimating catch-at-age.

In fitting the parameters of the model to observed catch-at-age data, it is necessary to have some measure of goodness of fit. Ideally this will be some function that weights the residuals using criteria derived from the probability distributions of the data. Therefore it is necessary to either specify or assume probability distributions for the estimation errors in these data. In most cases where separable virtual population analyses have been applied, least squares criteria have been used on log transforms of the catch-at-age estimates which is consistent with maximum likelihood estimation where errors are log-normally distributed (Doubleday 1976; Paloheimo 1980; Pope and Shepherd 1982). Fournier and Archibald (1982), on the other hand, identified two sources of error in estimating catch-at-age. They assumed that estimates of total catch were log-normally distributed, but that estimates of the proportion of the total catch in each age-class followed a multinomial distribution. Their objective function was based on the likelihood function of estimates of total catch and the proportion of the catch in each age-class.

This paper adapts separable virtual population analysis techniques to the complex life history of Pacific salmonids, and presents a method for dealing with uncertainty in the error structure of data. The techniques developed are applied to marked chinook salmon, *Oncorhynchus tshawytscha*, from hatcheries in California's Central Valley, and some management implications of the results are discussed.

Methods and Data

In the case of California's chinook salmon, the classical catch equations are an inadequate description of catch and survival. Commercial and sport troll fisheries both harvest substantial numbers of chinook salmon from California's coastal waters. These two fisheries have different minimum size limits, and fishing effort varies independently in the two fisheries. In addition to natural mortality and fishing mortality there is also mortality associated with spawning, and fishing is seasonal rather than continuous. Some mortality also results from the catch and release of sublegal fish, called shakers, which is inflicted primarily by the commercial troll fishery on age 2 and age 3 fish. This mortality is assumed to occur prior to recruitment and is omitted from this analysis. Omission of shaker mortality biases recruitment estimates downward and may lead to slight overestimation of the availability of age 2 and age 3 fish, but the magnitude of shaker mortality has never been adequately determined in field studies, and cannot be estimated reliably by the model.

Most chinook salmon in California enter the ocean in their first year of life and reach the minimum legal size for the commercial fishery in their third year, with moderate numbers appearing in the sport catch (which has a lower minimum size limit) in their second year. The commercial fishing season usually opens sometime around the first of May and closes near the end of September. The sport fishing season opens in February and closes in November, but most of the fishing takes place in the summer months. The majority of fish mature and spawn at ages 3 and 4, with small numbers maturing at ages 2 and 5. Most maturing fish leave the ocean during the fishing season and all die after spawning.

Description of the life history of chinook salmon can be simplified by dividing each year into three time periods. If we start time at the beginning of the commercial fishing season and assume that sport fishing mortality can be adequately described by a fishing season that coincides with the commercial season, then there are three critical times during each year: t_0, the beginning of the year; t_1, the time when maturing fish leave the ocean fishery to spawn; and t_2, the end of the commercial fishing season. From t_0 to t_1, fish are subject to natural mortality and commercial and sport fishing mortality. At t_1, a fraction of the cohort leaves the fishery to spawn and the remainder are subject to natural mortality, commercial and sport fishing mortality from t_1 to t_2, and natural mortality from t_2 to t_0. If we let $F_{1,a,t}$ be the commercial fishing mortality rate for fish of age a in year t and $F_{2,a,t}$ be the sport fishing mortality rate, then

$$(1)\quad F_{1,a,t} = q_{1,a} f_{1,t}$$

$$(2)\quad F_{2,a,t} = q_{2,a} f_{2,t}$$

where $q_{1,a}$ and $q_{2,a}$ are the vulnerabilities of fish of age a to the commercial and sport fisheries, respectively, and $f_{1,t}$ and $f_{2,t}$ are commercial and sport fishing rates in year t. The total mortality rate during the fishing season is then given by

$$(3)\quad Z_{a,t} = F_{1,a,t} + F_{2,a,t} + M.$$

During the time interval from t_0 to t_1 the commercial catch from one cohort is

$$(4)\quad {}^{1}C_{1,a,t} = N_{a,t} F_{1,a,t} [1 - \exp(-Z_{a,t} t_1)]/Z_{a,t},$$

and from t_1 to t_2 the commercial catch is

$$(5)\quad {}^2C_{1,a,t} = N_{a,t} F_{1,a,t} (1 - P_a) \exp(-Z_{a,t} t_1) \times \{1 - \exp[-Z_{a,t} (t_2 - t_1)]\}/Z_{a,t}$$

where P_a is the probability of maturing at age a. After combining the catches for the two time segments and rearranging, total commercial catch becomes

$$(6)\quad C_{1,a,t} = N_{a,t} F_{1,a,t} [1 - P_a \exp(-Z_{a,t} t_1) - (1 - P_a) \exp(-Z_{a,t} t_2)]/Z_{a,t}.$$

Sport catch is then given by

$$(7)\quad C_{2,a,t} = N_{a,t} F_{2,a,t} [1 - P_a \exp(-Z_{a,t} t_1) -(1 - P_a) \exp(-Z_{a,t} t_2)]/Z_{a,t},$$

spawning escapement is given by

$$(8)\quad S_{a,t} = P_a N_{a,t} \exp(-Z_{a,t} t_1),$$

and survival of the cohort to the next year is given by

$$(9)\quad N_{a+1,\,t+1} = N_{a,t} (1 - P_a) \exp[-Z_{a,t} t_2 - M (1 - t_2)].$$

For the natural mortality rate (M), I used a value of 0.20 which appears reasonable based on general natural mortality rates of Pacific salmon (Ricker 1976).

In order to fit this model to a salmon population, we require catch-at-age estimates for the commercial and sport fisheries, escapement-at-age estimates, and an objective function. In California the catch and spawning escapement of chinook salmon are not routinely aged. I have therefore used a segment of the population which is routinely aged, namely marked fish from hatcheries in the Central Valley. Fish from Central Valley hatcheries have been marked intermittently since the 1958 brood year. In the 1950s through early 1970s, fish were marked with multiple fin clips, and from the late 1970s to the present, marking has been by coded-wire tags (Jefferts et al. 1963).

To develop an appropriate objective function, we need to examine the methods by which estimates of the numbers of marked fish in the catch and escapement are generated. The total commercial salmon catch is obtained from copies of the sales receipts from fish buyers, and the proportion of the catch composed of chinook salmon is estimated by a modified Hartly–Ross estimator from a sample of the catch (Abramson and Jensen 1963). A portion of the commercial catch is inspected by California Department of Fish and Game (CDFG) personnel for marks, and the number of marked fish landed is estimated by multiplying the number of marks recovered by the ratio of total catch to the number inspected. Separate estimates are made for each of five major port areas at semimonthly intervals throughout the season, so an estimate of marks recovered in a year is the sum of many independent estimates. The total ocean sport catch is estimated by a stratified sampling scheme of sport fishing boats in major sport fishing ports with the strata being weekdays and weekends (Lesh 1977). Samples of the sport catches are counted and inspected for marks. As in the commercial catch, separate estimates are made for each major sport fishing port on a semimonthly basis.

Because of the sampling scheme there are two sources of error in estimating the number of marked fish in the catch. The first results from estimating the total catch and the second from estimating the proportion of fish that are marked. By the central limit theorem for independent variables, the estimate of the number of ocean-caught (commercial or sport) marked fish from a specific brood year should have a probability distribution that is approximately normal with a variance equal to the sum of the variances of all the independent estimates that contribute to it. Since the probability distribution of the estimated catch-at-age is approximately normal, the likelihood function of the catch estimates can be approximated by

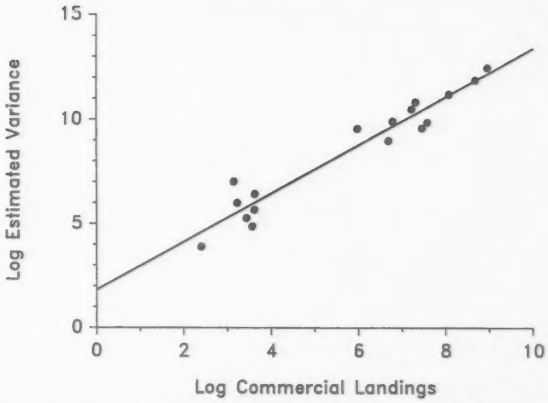

FIG. 1. Relationship between the size of the estimated catch at age and the variance of the estimates for commercially caught marked fish during 1970–76. The regression line has slope 1.2 and intercept 1.8 with $r^2 = 0.93$.

$$(10)\quad L = 1/\sqrt{2\pi}\sigma \prod_a \prod_t \exp[-(O_{a,t} - C_{a,t})^2/2\sigma^2]$$

where $C_{a,t}$ is the actual catch of fish of age a in year t, $O_{a,t}$ is the estimated catch of fish of age a in year t, and σ^2 is the variance of the estimate of catch of fish of age a in year t. Taking the negative of the log of the likelihood function and removing terms that are constant leaves

$$(11)\quad \Phi^* = \sum_a \sum_t (O_{a,t} - C_{a,t})^2/\sigma^2.$$

Unfortunately, estimates of the variances of the estimated catches of marked fish are unavailable for most years. However, it seems reasonable to assume that the variance of catch estimates would be related in some way to the size of the catch itself. The variances of commercial catch estimates of marked cohorts were estimated by CDFG for 1970–76 (L. B. Boydstun, CDFG, Region II, 1701 Nimbus Road, Suite B, Rancho Cordova, CA 95670, unpubl. data). The variances were estimated for each mark in each semimonthly period, assuming multinomially distributed errors, and then summed over each season and over each age-class. Using the results of regression of the log of estimated variance on the log of estimated catch (Fig. 1), the variances of commercial catch estimates may be approximated by

$$(12)\quad {\sigma_1}^2 = k_1 {C_1}^{b_1}$$

where $k_1 = \exp(1.8) = 6.0$ and $b_1 = 1.2$.

Estimates of total spawning escapement of Central Valley chinook salmon stocks are made by CDFG using a wide variety of information. Returns to the hatcheries are completely enumerated and the spawning escapements to individual streams are estimated separately. In some streams, modified Schaefer estimates are made using recapture data from tagged carcasses (Taylor 1974; Sykes and Botsford 1986). Smaller

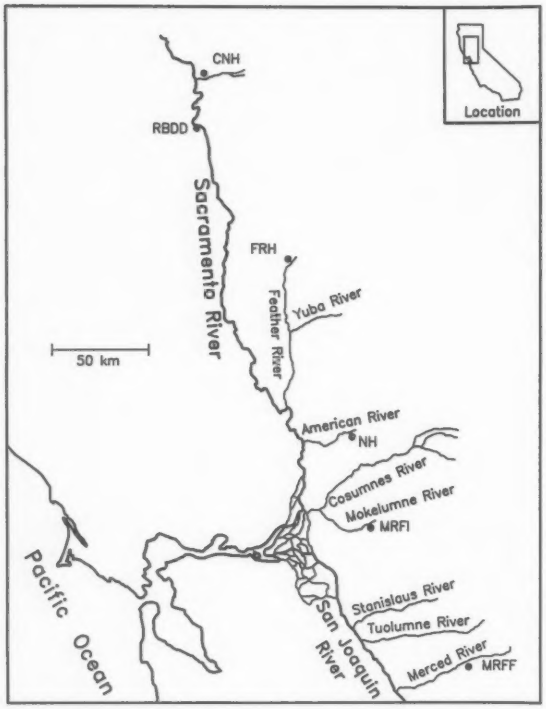

FIG. 2. Salmon spawning streams in California's Central Valley. For the sake of estimating the escapement of marked fish, the streams were divided into four regions as follows: Region 1, the main stem of the Sacramento River and all tributaries north of the Feather River; Region 2, the Feather and Yuba rivers; Region 3, the American River; Region 4, the San Joaquin River system including all streams from the Cosumnes River south. Facilities where fish are routinely examined for marks include Coleman National Hatchery (CNH), Red Bluff Diversion Dam (RBDD), Feather River Hatchery (FRH), Nimbus Hatchery (NH), Mokelumne River Fish Installation (MRFI), and Merced River Fish Facility (MRFF).

streams are surveyed periodically by CDFG personnel and the escapement is estimated by counting redds and carcasses and estimating the percentage of redds and carcasses counted. In the main stem of the Sacramento River, aerial redd counts and counts of salmon made at Red Bluff Diversion Dam over closed circuit television are combined to estimate total escapement. Estimates of the total escapement to the entire Central Valley are made by adding the estimates for all the rivers and creeks to the counts made at hatcheries and spawning channels.

Although fish returning to hatcheries have always been inspected for marks, there has been no attempt to systematically determine the numbers of marked fish in the total spawning escapement. Since 1978 the fresh carcasses recovered in the stream surveys have been inspected for marks and the numbers of marked fish recovered at hatcheries and in the stream surveys have been compiled (Anadromous Fisheries Branch, CDFG, Fish and Game, Region II, 1701 Nimbus Road, Suite B, Rancho Cordova, CA 95670, unpubl. data). In order to estimate the total escapement of marked fish, I divided the Central Valley into four regions, with each region containing at least one facility where fish were inspected for marks (Fig. 2). I estimated the number of marks in each region for each year by multiplying the number of marks recovered by the ratio of total spawners to the number of fish inspected. I then added the estimates for all regions and all marks of the same age to generate escapement-at-age estimates. Marks recovered at the hatchery of origin were excluded from the calculation of regional estimates, but were added to the estimates of total escapement. For example, suppose a particular mark was given to a release from the Feather River Hatchery. When these fish returned to spawn at age 3, 10 000 fish returned to the Feather River Hatchery of which 350 bore that mark, and an estimated 30 000 fish spawned in the remainder of the Feather River system of which 10 000 were inspected for marks and 50 marks were recovered. Then the estimated escapement of that mark for the Feather River would be $350 + 50\,(30\,000/10\,000)$, or 500 fish. If on the American River, 15 000 spawners returned to Nimbus Hatchery and 20 marks were recovered at the hatchery, and in the river, 20 000 fish were estimated to have spawned of which 5000 were inspected for marks and 5 marks were recovered, then the estimate for the number of marked fish spawning in the American River would be $(20 + 5)\,(15\,000 + 20\,000)/(15\,000 + 5000)$, or 44 fish.

Because of the variety of methods used to estimate Central Valley spawning escapement and the fraction of total escapement consisting of marked fish, the probability distributions of the estimates of spawning escapement of marked broods of fish are not known. For the sake of convenience, I have assumed that the estimation errors for sport catch and spawning escapement are related to the estimates themselves in a manner similar to the estimation errors for the commercial catch, but that the relative magnitudes of the errors may differ among the three classes of data. I used an expression similar to equation (12) for each of the three classes of data, commercial catch, sport catch, and spawning escapement, and estimated the coefficients using maximum likelihood from the residuals resulting from an unweighted least squares fit of the model to the data. There was little change in the value of the likelihood function over a broad range of values for the exponents when the function was maximized with respect to the coefficients. For this reason, the exponents were all set equal to 1.2 and only the coefficients were estimated.

Given the assumption that the variance of errors is proportional to the estimate raised to a power, the objective function for catch and escapement can be written as

$$(13)\quad \Phi = \sum_a \sum_t (O_{1,a,t} - C_{1,a,t})^2/k_1\, C_{1,a,t}{}^{b_1} + (O_{2,a,t} - C_{2,a,t})^2/k_2\, C_{2,a,t}{}^{b_2} + (E_{a,t} - S_{a,t})^2/k_3\, S_{a,t}{}^{b_3}$$

where $S_{a,t}$ is the actual spawning escapement of fish of age a in year t, $E_{a,t}$ is the estimate of spawning escapement of fish of age a in year t, k_1, k_2, and k_3 are the coefficients for the variance estimates for commercial catch, sport catch, and spawning escapement, respectively, and b_1, b_2, and b_3 are the exponents for these variance estimates. In this objective function the values of these exponents determine the relative weights assigned to small versus large observations, while the coefficients reflect the relative weights assigned to the different types of data. The actual values of the coefficients are irrelevant to the fitting procedure, but their relative magnitudes determine how heavily residuals from commercial catch, sport catch, and spawning escapement are weighted.

TABLE 1. Observed and predicted commercial catch values. Each observed value is the sum of all marked fish of the same age used in the analysis. Missing data are indicated by a dash.

	Observed catch-at-age				Predicted catch-at-age			
Year	2	3	4	5	2	3	4	5
1969	0				0			
1970	11	3188			10	3192		
1971	42	1937	1721		49	2040	1589	
1972	35	7133	798	13	51	7432	631	96
1973	—	7709	1814	0	—	7486	1930	31
1974	—	—	1350	41	17	—	1315	59
1975	0	876	—	23	5	861	—	19
1976	25	877	391	—	22	955	368	—
1977	0	2872	93	0	39	2754	213	15
1978	12	3427	331	17	47	3374	377	5
1979	184	4542	536	0	112	4662	555	11
1980	13	13270	551	0	91	12890	701	15
1981	85	6155	709	16	106	5820	761	7
1982	125	9911	989	6	92	10159	766	16
1983	0	4308	225	13	33	4411	433	6
1984	9	3016	778	0	13	3147	701	12

TABLE 2. Observed and predicted sport catch values. Each observed value is the sum of all marked fish of the same age used in the analysis. Missing data are indicated by a dash.

	Observed catch-at-age				Predicted catch-at-age			
Year	2	3	4	5	2	3	4	5
1969	40				40			
1970	263	1953			288	1920		
1971	2535	1504	488		2069	1820	637	
1972	822	4656	194	0	1401	4281	163	28
1973	—	1234	155	0	—	1228	142	3
1974	—	—	90	0	119	—	86	4
1975	99	217	—	0	70	256	—	3
1976	99	124	124	—	310	286	50	—
1977	314	534	14	0	347	516	18	1
1978	905	885	43	0	720	1083	54	1
1979	1931	2742	86	0	2546	2228	119	3
1980	141	2281	58	0	673	1987	49	1
1981	793	645	41	0	650	747	44	0
1982	1387	1271	53	0	925	2131	72	2
1983	74	1329	26	4	442	1244	55	1
1984	288	1213	69	0	247	1210	121	2

To fit the parameters of the cohort model to the observed catch and escapement data, the equations for catch and escapement were substituted for $C_{1,a,t}$, $C_{2,a,t}$, and $S_{a,t}$ into equation (13) and a minimum value of the objective function was sought. This was accomplished with a steepest descent algorithm. From initial estimates for all parameters, the partial derivatives of the objective function were evaluated at the initial values. All parameters were then adjusted in the direction indicated by the values of their partial derivatives and the process was repeated until changes in the values of the parameters failed to reduce the value of the objective function. There is no guarantee that the set of parameter values arrived at by this procedure will provide a unique best fit to the observed data, but numerous runs with simulated data sets have produced consistent results. It should be noted that since effort and vulnerability appear in the model only as a product, unique solutions of these parameters cannot be obtained. It is always possible to multiply the values of effort by a constant and divide the values of vulnerability by the same constant without altering the force of fishing mortality or consequently the value of the objective function. Aside from this problem of scaling, this method of fitting the parameters to a data set appears to be quite robust. Solutions with clean simulated data consistently match the parameter values used to generate the data, and solutions for multiple runs with simulated data containing errors have been equivalent regardless of the initial parameter estimates used.

TABLE 3. Observed and predicted escapement. Each observed value is the sum of all marked fish of the same age used in the analysis. Missing data are indicated by a dash.

	Observed escapement-at-age				Predicted escapement-at-age			
Year	2	3	4	5	2	3	4	5
1969	—				1311			
1970	—	—			541	6742		
1971	—	—	—		1426	2146	4662	
1972	—	—	—	—	998	4964	1159	108
1973	—	—	—	—	—	3283	2231	21
1974	—	—	—	—	210	—	1372	36
1975	317	350	—	—	147	963	—	37
1976	475	521	590	—	344	513	531	—
1977	470	1059	127	5	464	1050	213	8
1978	398	1374	271	8	614	1402	420	3
1979	883	832	708	0	1205	1433	467	5
1980	147	1802	484	1	610	2109	294	3
1981	940	881	360	2	960	1536	518	2
1982	479	2911	196	0	622	1649	324	3
1983	644	1319	268	0	445	1950	512	4
1984	144	1577	224	0	178	1312	789	8

The data used in this analysis (Tables 1, 2, and 3) have a number of missing values. The first four cohorts that passed through the fishery were marked with multiple fin clips, and the inland recoveries of these fin clips were not reported adequately to generate estimates of the same accuracy as the estimates for coded-wire tags, so these estimates were omitted. In the 1971 brood year, no fish were marked, and in 1974, coded-wire tag recoveries were not reported. The data from multiple fin clips cannot be considered as reliable as data from coded-wire tags. Clipped fins can regenerate and physical injuries can result in false marks. In addition, there was some redundancy in the marks issued by Central Valley hatcheries for the four cohorts of fin-clipped fish included in this analysis. With simulated data sets, the predicted catch and escapement provided better estimates of the actual values than the observed catch and escapement when random observational errors were included. With the real data, for reasons discussed below, calculated values cannot be considered more accurate than the observed values.

Results and Discussion

There is generally good agreement between predicted and observed values of commercial catch (Table 1), sport catch (Table 2), and spawning escapement (Table 3). However, in all three classes of data, the median weighted residual is negative (Table 4). This is due primarily to a preponderance of negative residuals in age-classes with low observed values (i.e. ages 2

TABLE 4. Weighted residuals for catch and escapement. Residuals were weighted by dividing by the standard deviation from equation (12), using the parameters from Table 6. Missing values are indicated by a dash.

	Commerical catch				Sport catch				Spawning escapement			
Year	2	3	4	5	2	3	4	5	2	3	4	5
1969	−0.0	—	—	—	−0.0	—	—	—	—	—	—	—
1970	0.1	−0.0	—	—	−0.2	−0.9	—	—	—	—	—	—
1971	−0.3	−0.4	0.6	—	1.2	−0.9	−0.8	—	—	—	—	—
1972	−0.6	−0.6	1.4	−2.1	−1.9	0.6	0.4	−1.0	—	—	—	—
1973	—	0.4	−0.5	−1.6	—	0.0	0.2	−0.4	—	—	—	—
1974	—	—	0.2	−0.6	—	—	0.1	−0.5	—	—	—	—
1975	−0.7	0.1	—	0.3	0.6	−0.4	—	−0.4	1.6	−1.7	—	—
1976	0.2	−0.5	0.3	—	−1.8	−1.4	1.9	—	0.7	0.0	0.2	—
1977	−1.7	0.4	−1.9	−1.2	−0.3	0.1	−0.2	−0.3	0.0	0.0	−0.6	−0.2
1978	−1.4	0.2	−0.5	1.8	0.9	−0.8	−0.3	−0.2	−0.8	−0.1	−0.7	0.5
1979	1.7	−0.3	−0.2	−1.0	−1.4	1.3	−0.5	−0.4	−0.8	−1.3	1.1	−0.4
1980	−2.1	0.5	−1.2	−1.2	−2.8	0.8	0.2	−0.3	−1.8	−0.5	1.2	−0.2
1981	−0.5	0.7	−0.4	1.1	0.8	−0.5	−0.1	−0.2	−0.1	−1.4	−0.7	−0.1
1982	0.9	−0.4	1.6	−0.7	2.0	−2.2	−0.4	−0.3	−0.5	2.5	−0.7	−0.4
1983	−1.6	−0.3	−2.2	1.0	−2.5	0.3	−0.7	0.9	0.9	−1.1	−1.0	−0.4
1984	−0.4	−0.4	0.6	−1.1	0.4	0.0	−0.8	−0.4	−0.3	0.6	−1.8	−0.5

TABLE 5. Age-specific parameter values providing the best fit to the observed data with a natural instantaneous annual mortality rate of 0.20. Vulnerabilities have been scaled so that the most vulnerable age-class in each fishery has a value of 1.0.

	Age 2	Age 3	Age 4	Age 5
Troll vulnerability	0.0045	1.0000	1.0101	1.0384
Sport vulnerability	0.2161	1.0000	0.4550	0.5194
Spawning probability	0.0600	0.4111	0.9335	1.0000

TABLE 6. Variance parameters used for weighting residuals in the fitting procedure. Exponents were set equal to 1.2 and the coefficients were estimated by maximum likelihood from the residuals of an unweighted least squares fit.

	Coefficient	Exponent
Troll catch	6.4	1.2
Sport catch	14.8	1.2
Spawning escapement	16.7	1.2

and 5 in commercial catch, ages 4 and 5 in sport catch, and age 5 in spawning escapement). This suggests that the normal approximation for observational errors does not hold for these small values. The individual estimates that comprise each of these observed values have such skewed distributions, with the majority of the estimates being zero, that their sum is not approximately normal. The mean of the residuals is also negative, which suggests that the parameter estimates may be slightly biased, but because of the complexity of the problem, I have not attempted to evaluate the possible bias.

Over the 16-yr period for which data are available, the force of fishing mortality exerted on fully recruited age-classes (ages 4 and 5) by the sport fishery has averaged about 12% of that exerted by the commercial fishery. It appears that both fisheries are targeting on 3-yr-old fish. Length frequencies of fish from the commercial and sport fisheries (R. Kope, unpubl. data) suggest that somewhat less than half of the fish in this age-class are above the minimum legal size for the commercial fishery. In spite of this, they appear to be just as vulnerable to the commercial fishery and about twice as vulnerable to the sport fishery as the older age-classes (Table 5). This could be caused by gear selectivity or by the spatial distribution of different age-classes. Fishermen may be using gear that is most effective on the most abundant sizes of fish, or younger age-classes may be more common in waters that are frequently fished. It is also possible that salmon travel in schools that are formed when they migrate to sea, resulting in uniform-aged schools. Fishermen would tend to concentrate their effort on larger schools, which would consist of younger age-classes, since mortality and maturation reduce the size of schools as the fish age.

Because of the large ratio of parameters estimated to the number of observations, the model can find solutions which fit observed data more closely than the actual values of the parameters being estimated. In runs with simulated data, the variances of observational errors were typically underestimated by about one half. In the analysis presented here, the maximum likelihood estimate of the variance coefficient for commercial catch (Table 6) is larger than the coefficient determined by regression from independent variance estimates. It seems likely, then, that violations of the assumptions of the model contribute at least as much to the unexplained variability in the observed data as to observational errors.

The most likely assumptions to be violated are the constancy of some of the parameters. For example, the natural mortality rate almost certainly varies with the size of fish and consequently with age. These age-specific rates probably vary from year to year under the influence of a number of biotic and abiotic environmental factors, but it seems unlikely that nonconstant natural mortality rates would contribute substantially to lack of fit, since recruitment and fishing mortality rates could be adjusted to compensate for most of the variability in natural mortality. A much more likely source of error is the assumption of year-to-year constancy in the vulnerabilities and spawning probabilities of the different age-classes. It would be difficult for this fitting procedure to compensate for violations of these assumptions by adjusting other parameters. Variability in the vulnerabilities could result from changes in the spatial distribution of fish or from changes in growth rate. Changes in the growth rate would affect the time at which fish reached the minimum legal size and would consequently exert its greatest

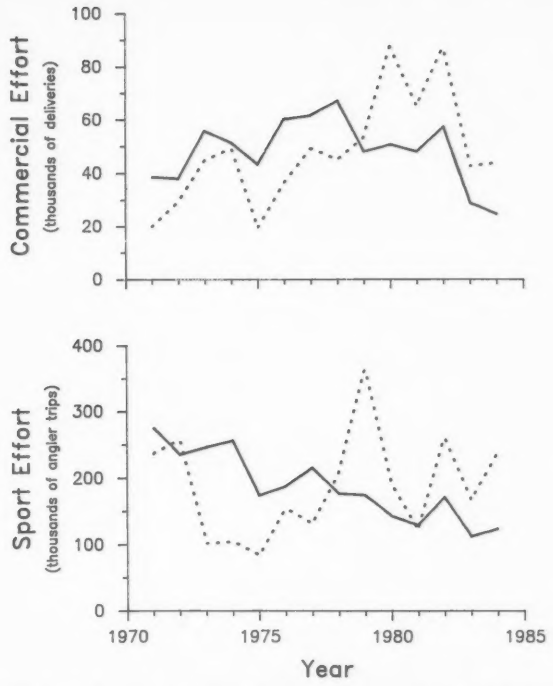

FIG. 3. (a) Commercial salmon fishing effort in California. The solid line is nominal effort as reported by CDFG and the broken line is effort as calculated by the model. Calculated effort has been rescaled so that the means of calculated and nominal effort are equal. (b) Sport salmon fishing effort in California. The solid line is nominal effort as estimated by CDFG and the broken line is effort as calculated by the model after rescaling as in Fig. 3a.

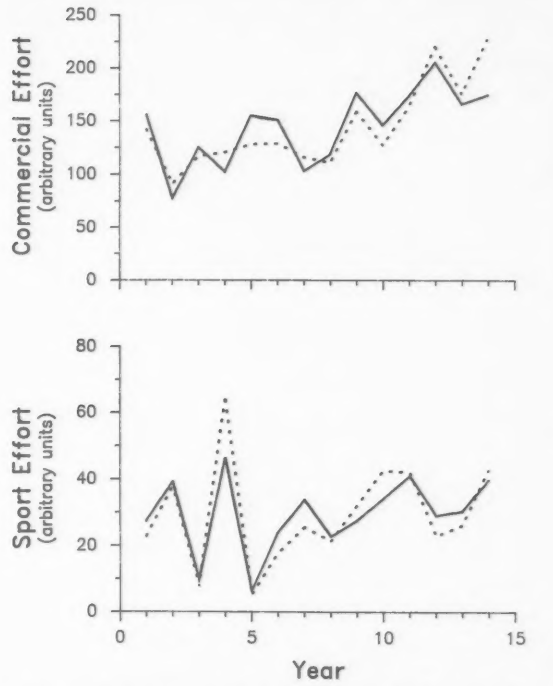

FIG. 4. (a) Simulated commercial effort. The solid line is actual simulated commercial effort and the broken line is effort as estimated by the model from data that contained simulated random observational errors and missing values as in the real data in Table 1. (b) Simulated sport effort. The solid line is simulated effort and the broken line is effort as estimated by the model from the same data set used for commercial effort in Fig. 4a.

effect on partially available age-classes. This could be reflected in larger residuals for partially available age-classes and should be most pronounced in the sport catch of age 2 fish. Though the process of fitting the model to the data tends to distribute variability in the magnitude of residuals among all age-classes, the sport catch of age 2 fish does appear to have the largest residuals. Changes in the spatial distribution of fish would probably affect the vulnerability of all age-classes simultaneously, and in this case, adjustments in estimated fishing effort could compensate perfectly.

Examination of the patterns of commercial and sport fishing effort (Fig. 3) reveals several points of interest. Nominal effort estimates made by CDFG have been in terms of numbers of deliveries and (since 1979) in days fished for the commercial troll fishery and in angler trips for the sport fishery (PFMC 1983, 1985). Realized fishing effort in both cases appears to be more variable than the nominal fishing effort reported by CDFG. This may be due in part to the errors in estimating catch and escapement, but is probably contributed to by nonconstant vulnerabilities. Effort estimates from simulated data with comparable observational errors (Fig. 4) show less discrepancy between actual effort and effort as estimated by the model, but with simulated data, none of the assumptions of the model are violated. In addition, there is a fundamental difference between actual effort in simulated data and nominal effort in the real fisheries. Nominal effort is estimated for the sport fishery, thus introducing additional observational errors, and in both fisheries, effort is reported in units that are only an approximation of realized effort. Clearly, all boats do not have the same fishing power and larger boats tend to fish longer than smaller boats before making deliveries. Year-to-year changes in fleet composition can influence the effect of an average unit of nominal effort. Therefore the realized effort estimated by the model may be a better estimate of the fishing pressure experienced by the salmon stocks than the number of deliveries, number of days fished, or number of angler trips. The last point of interest regarding Fig. 3 is that realized effort in both fisheries appears to be increasing relative to nominal effort. While nominal effort has been relatively stable in the commercial fishery, realized effort has been increasing, and while nominal effort in the sport fishery has been decreasing, realized effort again appears to have been increasing in recent years. Apparently, vulnerability of salmon to both fisheries is increasing or fishermen are getting better at catching fish. Although I am not aware of any improvements in the terminal tackle of salmon fishermen that could account for increased fishing power, improvements in fish-locating technology and increased cooperation among fishermen could account for the observed increase in the effectiveness of effort.

This increase in the effectiveness of fishing effort coincides with measures implemented by managers to try to reduce fishing mortality rates in the commercial fishery. In recent years there have been in-season closures to limit the commercial catch to quotas established by the Pacific Fishery Management

Council while spreading the catch over the entire commercial fishing season. California has also implemented a limited entry program for the salmon fleet. As a result of the more restrictive management practices, fishermen may be fishing longer hours for each landing, and less successful fishermen may be leaving the fleet. Regardless of the cause, the result of the increased effectiveness of effort is to reduce the impact of regulations designed to decrease fishing mortality by limiting effort.

To the degree that the marked fish used in this analysis are representative of the Central Valley stock, the fishing mortality rates and maturity schedules estimated by the model reflect those of Central Valley fish in general. Recruitment as estimated by the model bears no relation to recruitment in the total stock, and is not reported here. By using the harvest rates and spawning probabilities determined from marked fish, it may be possible to generate estimates of recruitment in the Central Valley stock, but these estimates would probably be very inaccurate. The estimates of fishing effort, vulnerability, and probability of maturation could be greatly improved, and estimates of recruitment from the Central Valley stock could be generated, if estimates were available of the age composition of the entire catch and spawning escapement. These estimates could be added to the end of the data matrix and the analysis extended to provide estimates of the abundance of fish age 3 and older to the fisheries prior to the opening of the fishing season. Such estimates would be invaluable to managers in establishing catch quotas that allow for adequate spawning escapement.

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An Egg-Mass Census Method for Tracking Fluctuations in Yellow Perch (*Perca flavescens*) Populations

G. E. (Buck) Newsome

Department of Biology, St. Francis Xavier University, Antigonish, N.S. B2G 1C0

and S. K. Aalto

Department of Mathematics, St. Francis Xavier University, Antigonish, N.S. B2G 1C0

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Extreme annual fluctuations among yellow perch (*Perca flavescens*) populations are common and have been related to changes in the physical environment during early stages of the development of a year-class. However, critical investigations of these relationships are often hampered by the lack of accurate techniques for estimating abundance. In this paper an egg-mass census technique is described, and statistical procedures which provide population estimates with confidence limits are given. Also discussed are results of preliminary investigations of environmental factors which may have a dramatic affect on the year-class strengths of perch. Winds play an important role in determining recruitment rates. Wind-generated internal seiches may result in thermal shocking of perch embryos which subsequently affects juvenile survival.

Les fluctuations annuelles extrêmes des populations de perchaudes (*Perca flavescens*) sont des phénomènes courants que l'on a liés à des variations de l'environnement physique survenant au cours des premières étapes du développement des classes annuelles. L'étude détaillée de ces relations est souvent limitée par l'absence de techniques précises d'estimation de l'abondance. Les auteurs décrivent une technique d'inventaire par masses d'oeufs et présentent des méthodes statistiques qui permettent d'obtenir des estimations de population avec des limites de confiance. Ils traitent aussi des résultats d'une étude préliminaire de facteurs environnementaux qui peuvent avoir des effets extrêmement importants sur le volume des classes d'âge chez la perchaude. Les vents influent fortement sur les taux de recrutement. En effet, les seiches produites par le vent peuvent être la cause de chocs thermiques pour les embryons et ainsi nuire à la survie des juvéniles.

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It is frequently reported that populations of yellow perch (*Perca fluviatilis* in Eurasia; *P. flavescens* in North America) exhibit very large annual variations in numbers and in year-class strengths (Doan 1942; Jobes 1952; el-Zarka 1959; Heyerdahl and Smith 1971; Forney 1971; Clady 1976; Craig et al. 1979; Treasurer 1983). Some researchers suggest that meteorological events which affect the aquatic environment play an important role in the large variation in year-classes which subsequently affects annual abundance. The effects of temperature variations and wind on various stages in the early development of perch seem to be the most frequently reported factors (Jobes 1952; Pycha and Smith 1955; Busch et al. 1975; Clady 1976; Eshenroder 1977; Koonce et al. 1977; Treasurer 1983). Other researchers suggest that biological factors such as predation and cannibalism on early life stages may result in the production of strong year-classes either directly or in concert with environmental conditions (Forney 1971, 1977; Noble 1971; Neilsen 1980). As Clady (1976) and Treasurer (1983) pointed out, critical research dealing with factors which may influence survival of early stages is, on the whole, sparse. We feel that a contributing factor behind the lack of any resolution to this question is the relative paucity of long-term investigations, with the notable exceptions of the work of Forney (1971), Clady (1976), Le Cren et al. (1977), and Craig et al. (1979).

In this paper we discuss our methodology and the early results of our own continuing long-term study, begun in 1978, of the perch population of Lochaber Lake, N.S. We present a method for estimating the number of female yellow perch based on a census of perch egg-masses and a method for estimating the parameters of a statistical model for egg-mass distribution. From this model, confidence intervals for the estimated number of egg-masses and therefore mature female perch are derived. From this analysis and other evidence, we develop mechanisms which relate meteorological events and survival rates of juveniles in an attempt to explain the large annual variations in perch numbers. The statistical evidence also provides indirect support for the demic behaviour of perch populations. Such behaviour may also contribute substantially to the large annual variation in perch populations.

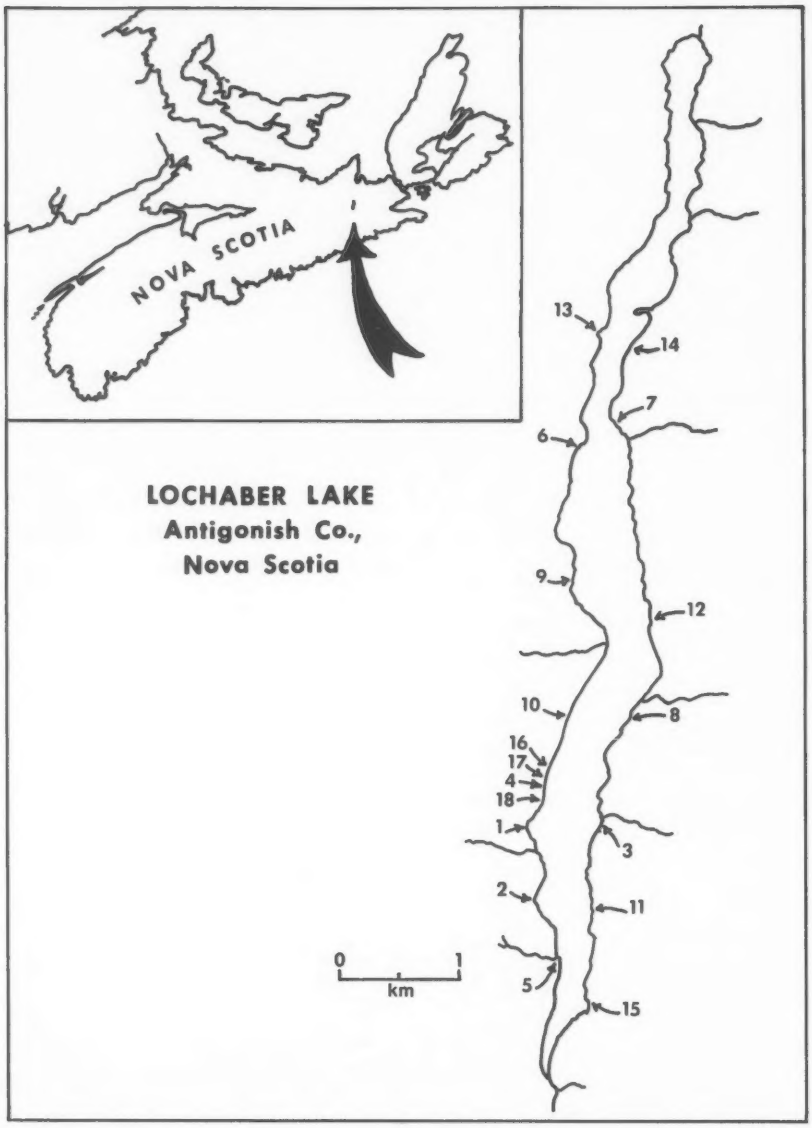

FIG. 1. Geographic location of the study lake and sample sites used throughout this investigation.

Materials and Methods

Study Lake

Lochaber Lake, Antigonish Co., Nova Scotia, is a moderately large lake of the graben type (Type 9, Hutchinson 1957) (Fig. 1; Table 1). It is surrounded by rolling hills covered in equal proportions of mixed forest, stands of pure second growth black spruce, and a variety of agriculture, predominantly pasture and strawberry fields. Bottom composition is primarily of glacial till, except in shallow sheltered bays where the bottom is composed of sand or mixtures of sand and fine silt. A cobble bottom predominates interspersed among large patches of loose stony rubble and smaller patches of larger stones and small boulders (10–30 cm diameter). Aquatic vegetation is very sparse and is restricted to the shallow bays at the north and south ends of the lake. Around the margins of the lake the dominant emergent plant is *Eriocaulon* sp. to a depth

TABLE 1. Geographic and limnologic features of Lochaber Lake, N.S. (45°25′N, 62°02′W).

Drainage system: St. Mary's River	Drainage area: 7018.1 ha
Surface elevation: 46 m	Surface area: 307.2 ha
Maximum length: 8440 m	Maximum width: 730 m
Maximum depth: 52.4 m	Mean depth: 21.8 m
Volume: 669 × 10^5 m^3	Shoreline development: 3.0
Conductivity: 46.6 μmhos/cm	pH: 6.5–7.5
Secchi disc depth: 3–5 m	Morphoedaphic index: 1.6

of approximately 0.5 m. From a depth of approximately 1 to 4 m, large carpets of *Isoetes* sp. are common, particularly where the bottom composition is of mixed rubble.

The lake is extremely oligotrophic and during the summer well stratified. A thermocline is first detectable around the middle of June (12–20) and well defined at a depth of approximately 10 m from late June until late October.

Egg-Mass Census

Yellow perch are unique among perciform fishes in that each mature female lays a single large gelatinous egg-mass annually. Egg-masses are slightly denser than water and rest on the bottom or are entwined among vegetative debris (Breder and Rosen 1966; Scott and Crossman 1973; Dorr 1982; Treasurer 1981, 1983). Because perch egg-masses are structurally distinct and readily observed, their abundance is a useful index of the population of mature females as first suggested by Zhakov (1962, 1964) and subsequently used by a number of other investigators (Menshutkin et al. 1968; Holcik 1969; Cerny and Pivnicka 1973; Aalto and Newsome 1980; Viljanen and Holopainen 1982; Treasurer 1983).

Since 1978, annual censuses of the distribution of perch egg-masses have been conducted at a number of fixed sample sites (Fig. 1). With two exceptions, sample sites were chosen at random beginning with 11 sites in 1978. The number of sample sites was increased over the years to 18 in 1983. Sites 17 and 18 are the two nonrandomly chosen sites. These two sites were chosen because the distances between Sites 4 and 16 and between Sites 1 and 4 were small enough to easily form two sites which could be combined to yield a larger sampling region with five subdivisions (Sites 1, 18, 4, 17, and 16, respectively).

Most investigators report that perch spawn in very shallow water (usually <3 m) and that egg-masses can be counted from the surface (Treasurer 1983). The perch population of Lochaber Lake, however, has never been observed to spawn at depths less than 3 m; rather, egg-masses are deposited between 4 and 14 m with the maximum number between 6 and 10 m. These observations are more consistent with those of Dorr (1982) for Lake Michigan perch. Thus, we developed a transect sampling technique, using SCUBA, which gives reliable results and is described below.

Each site was permanently marked by polypropylene rope (0.6 cm diameter), fixed to the bottom with large spikes or rocks. The ropes were laid perpendicular to the slope from a depth of 3 to 14–15 m. Counts of egg-masses were performed by two SCUBA divers, each swimming two isobathic transects. Transects at depths 6, 8, and 10 m were counted at all sites along with one chosen from either the 4 or 12 m depth. In June 1986, divers estimated the area sampled during a transect by measuring distances at which egg-masses were first perceived. This distance was 1 m for both divers and the area sampled was computed by multiplying transect lengths by 2.

Site counts were begun upon termination of spawning (June 16–23) and completed within 5–6 d. The date on which spawning was complete was determined by counting the egg-masses laid at Site 1 at regular intervals beginning in early June (June 1–5). Completion of spawning was indicated when the number of egg-masses observed at Site 1 ceased to increase. Since egg-masses break up slowly only after larvae hatch, and incubation requires approximately 20 d, all egg-masses laid on transects during the spawning period can be counted.

Treasurer (1983) observed little damage to egg-masses by invertebrate predators. Newsome and Tompkins (1985) have demonstrated that egg-masses are protected in some chemical fashion from a wide variety of potential teleost and invertebrate predators. More recently, Diamond and Wakefield (1986) have clearly demonstrated that few invertebrates will consume perch eggs, and thus, it was unnecessary to correct the counts of egg-masses for any losses due to predation.

Fish Collections

Estimates of female age-specific fecundity and survival rates were made from samples of perch collected using unbaited "Windermere traps" (Worthington 1950; Bagenal 1972) set at depths of 4–10 m and emptied every 24 h. Gravid females for fecundity analysis were trapped from Sites 1, 2, and 4 during late May and early June in 1976, 1978, and 1980. Fish were measured (total and standard length to the nearest millimetre), weighed (nearest 0.1 g), ovaries extracted and weighed (nearest 0.01 g), and scales removed from the third row below the lateral line below the insertion of the first dorsal spine. Age determination was made from microscopic inspection of scales mounted between microscope slides taped together. Extracted ovaries were subsampled in triplicate (approximately 0.1 g each), number of ova counted, and the mean used to estimate respective fecundity. Samples were pooled (n = 55) to provide an estimate of the population's age-specific fecundity (GM-regression, Ricker 1973).

Collections for the estimation of age-specific survival were made annually (except 1983) from various sample sites during the spawning period (June 10–25). Separate collections of single scales, removed from the same location described above, were made from both sexes and used to estimate annual age distributions. Captured fish were returned to the population.

Site Surveys

During the summers of 1983–84 the 18 sample sites were accurately surveyed, isobathic charts prepared, and estimates of area between depths of 4–12 m computed using a planimeter. The area sampled by divers was compared with the total area under 4–12 m of water for each site as a percentage (Table 2). Bottom composition at each site was scored according to the dominant substrate material present (silt = 1 to boulder = 7) based on a modified Wentworth scale (Wentworth 1920) as reported by Dorr (1982). Mean slopes at each site were determined from three perpendicular transects measuring depths at 2-m intervals between depths of 4–15 m. The transects were made at the ends and middle of each sample site and the mean slopes for each transect were averaged to obtain the mean slope for each site.

Dorr (1982) reported that perch in Lake Michigan selected rough substrates for spawning and hypothesized that this might be related to currents. Substrate with rough profiles would provide crevices and snags which would trap egg-masses and thus reduce the likelihood that they might be dislodged as was reported by Clady and Hutchinson (1975). In 1983, drift bottles attached to hardware-cloth drogues on 8-m lines were released on calm days. The track of the bottles was plotted and timed to give a crude indication of major circulation patterns of the surface waters of the lake. The effect of current on the selection of sites by female perch in 1984 was surveyed at each site by estimating the current near the bottom at 8 m. This

TABLE 2. Morphological features of sample sites used in annual census counts of yellow perch egg-masses, Lochaber Lake, N.S.

Site No.	Transect lengths at depths (m) 4	6	8	10	12	Total area (m^2)	Percent of area censused	Mean slope	Bottom composition (substrate score)
1	108	108	108	112	120	8 240	2.8	0.19	Mixed cobble and boulder (6)
2	184	192	224	184	176	17 284	3.2	0.14	Fine sand (2)
3	120	120	120	115	110	3 736	9.3	0.52	Mixed cobble (5)
4	100	100	100	104	100	5 776	5.3	0.37	Mixed cobble and boulder (6)
5	80	76	76	64	60	3 632	5.6	0.35	Mixed cobble and silt (3)
6	156	156	152	156	164	8 784	5.4	0.34	Mixed cobble and silt (5)
7	72	68	62	56	52	3 952	4.6	0.38	Mixed boulder and cobble (7)
8	92	92	96	96	92	4 056	6.9	0.43	Mixed cobble (6)
9	112	112	120	120	120	7 016	5.1	0.33	Mixed boulder and cobble (7)
10	136	140	136	140	140	10 144	4.1	0.32	Mixed cobble and boulder (6)
11	96	96	104	92	96	4 352	6.7	0.46	Mixed pebble (5)
12	128	128	132	132	136	5 400	7.3	0.54	Mixed cobble (6)
13	92	96	96	92	96	3 480	8.1	0.41	Mixed cobble (6)
14	100	100	100	104	112	4 312	7.4	0.43	Mixed silt and pebble (2)
15	92	88	88	88	88	10 064	2.6	0.47	Uniform pebble (3)
16	140	136	136	136	132	8 448	4.8	0.38	Mixed boulder and cobble (7)
17	64	72	68	68	70	2 872	7.1	0.35	Mixed cobble (6)
18	176	176	172	170	170	7 320	7.0	0.33	Mixed pebble (5)

estimate was made by a modification of the plaster "clod-card" technique (Doty and Doty 1973). Approximately 25-g "clods" of "plaster of paris" formed in ice-cube trays were glued, using silicone cement, to lucite (2 mm thick) cards (10 × 5 cm). Before use, the clod-cards were dried to a constant weight in an oven at 100°C. Between June 15 and 22, 1984, five clod-cards, attached to standard bricks with heavy elastic bands, were placed at a depth of 8 m at the center of each sample site. After 48 h of exposure the cards were removed, dried in a dust-free atmosphere, and dried in an oven (100°C) until a constant weight was achieved. The mean net change in the weight of the clod-cards was used as an index of the amount of bottom current occurring at each site.

Weather Data

Annual June meteorological data, except wind, were obtained from the Collegeville Observation Station (No. 820-1000-190) located 3 km north of the lake. Hourly wind observations for June (direction and velocity) were obtained from the Eddy Point Station (Ref. code WOQ) between 1975 and 1984 and subsequently from the Hart Island Station (Ref. code WRN), both of which are located approximately 60 km east of Lochaber Lake.

These data were used to compute descriptive statistics that were used in correlation and multivariate regression analyses: (1) degree days = sum of daily mean June temperatures, (2) degree days above 10°C = sum of daily mean June temperatures greater than 10°C, (3) mean temperature in June, (4) warming rate index (over the first 15 d in June) = slope of the relationship between days in June and the sum of the corresponding running means of the mean daily temperature (Clady 1976), (5) daily sky-cover index (0 = clear to 4 = overcast with precipitation), (6) June sky-cover index = mean of daily sky-cover index, (7) total precipitation (millimetres) in June, (8) mean water temperature in Lochaber Lake at 8 m on June 15, (9) total wind velocity for June = sum of hourly mean velocities from the 16 cardinal compass directions, (10) wind velocities for June by quadrant = sum of hourly mean velocities from directions in each of the four quadrants, and (11) wind velocities for June by sector = sum of hourly mean velocities from directions in each of the six sectors.

Results and Discussion

Spawning begins in the last week of May or during the first few days of June and is completed in the period June 15–22. From the annual plots (Fig. 2), we estimate the date at which spawning had been completed. These annual dates (1978–85) were compared using correlation analysis with meteorological variables 1–8. Analysis revealed that only the index of the rate of warming (variable 4) over the first 15 d of June was significantly related to the annual date for the completion of spawning ($r^2 = 0.421$, $p = 0.041$); the amount of precipitation (variable 7) accounted for an additional 25% of the variation ($p = 0.123$) between the date of completed spawning and the remaining factors investigated. The fact that 25% of the explained variation in the date for the completion of spawning can be assigned to variation in the June precipitation is interesting and may be a subsidiary thermal effect. The resulting additional runoff in years of above average precipitation pre-

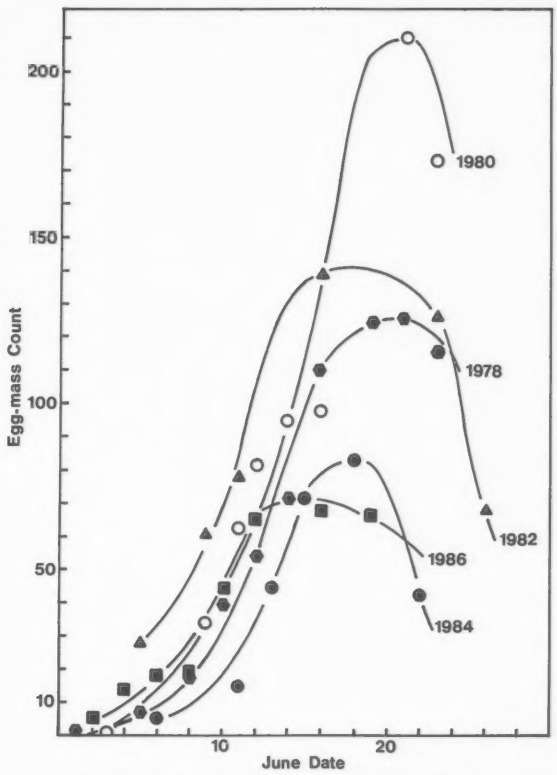

FIG. 2. Spawning progression for yellow perch, Lochaber Lake, N.S. (Site 1), determined from egg-mass census. Alternate years are plotted (June 1978–86).

sumably affects the rate of warming.

Table 2 gives the lengths of each transect by site and depth. The egg-mass counts made by the divers may be standardized to transects of 100 m in length by multiplying by the factor 100/(transect length). As noted above, SCUBA divers can observe egg-masses at a maximum distance of 1 m and thus can count all egg-masses in a 2-m-wide swath along each transect. Thus, if each of the diver's egg-mass counts is multiplied by this factor, then the result is a table of counts per 200 m^2. Table 3 reports this adjustment for all annual census results.

These data suggest that the density of egg-masses varies with depth and site. Egg-masses are more likely to be found at the 8-, 10-, and 12-m depths than at the others. Further, divers observed that egg-masses are more likely to be found in locations where the bottom is relatively flat and composed of smaller coarse gravel, and less likely to be found where the bottom is relatively steep, or flat and covered with fine silt. These observations are consistent with those reported by Dorr (1982). To discover which of the three factors, slope, current, or substrate composition, most affected the intensity to which perch utilized a particular site, correlation analysis was used to compare the sum of the number of egg-masses observed on isobathic transects for depths 6, 8, and 10 m and the following indices: (a) mean slope of each site, (b) index of current (from clod-card weight changes (Doty and Doty (1973), and (c) an index of substrate composition (Table 2). Only mean slope was significantly correlated with egg deposition accounting for 32% of the variation ($p = 0.008$); neither current ($r^2 = 0.007$, $p = 0.373$) nor substrate ($r^2 = 0.042$, $p = 0.217$) greatly influence the degree to which perch utilize a particular site. However, divers observed that slope and substrate composition are related: steep slopes were frequently covered with coarse cobble and boulders. Also, the substrate can vary widely within a single site and along individual transects. It is not surprising that substrate composition did not appear to affect significantly the frequency with which perch utilized particular spawning sites because in Lochaber Lake, there is little variation from site to site on a broad scale. However, Sites 1 and 4 are exceptions, each having relatively heterogeneous substrates. Particularly at Site 1, large patches of coarse glacial till are interspersed among patches of fine sand and silt. Our qualitative observations that female perch strongly select rough substrates agree with those of Dorr (1982). The fact that both Sites 2 and 15, which are the most homogeneous of our 18 sites, have very smooth surfaces and are used only weakly as spawning sites lends additional support to these observations.

Dorr (1982) suggested that the reason that perch select rough substrate for spawning may be related to the vulnerability of the egg-masses to movement by water currents. Clady and Hutchinson (1975) noted the displacement of large numbers of egg-masses following a severe wind storm during the reproductive period of Lake Oneida perch. Observations of the tracks of drift bottles showed the presence of two independent counterclockwise circulation currents, one located in the north basin and the other in the south. Estimates of their rates of flow indicate rates somewhat below 2 cm/s. Although somewhat crude, our results do not indicate that bottom currents are a major factor influencing spawning site usage by perch in Lochaber Lake. However, the influence of current on the choice of a spawning site by perch may be beyond the capability of our methods. More accurate technology for measuring currents below 2 cm/s is required to determine if current plays a significant role in spawning site selection by yellow perch.

Another way of considering the census data is to study the frequency that the various numbers of egg-masses are observed. As the density varies by site and depth, such examinations should be made by year and depth. An example is the frequency of egg-masses per 200 m^2 at 10 m in 1985 which is given below:

Frequency	0 1 2 3 4 5 6 7 8 9 10 11...19...36
No. of sites where observed	2 4 1 2 2 2 0 1 0 0 1 0 ...1 ...1

The mean density is 6.33 with a standard deviation of 9.05. This fact and the frequencies reported in the table above suggest that the data are not normal. Examination of the egg-mass data (Table 3) shows that many of the frequency tables derived from these data for various years and depths will have nonzero frequencies far from the mean.

The frequency tables and the above-mentioned observations of divers concerning the location of egg-masses suggest that the data may be better explained by a bimodal (or multimodal) probability model than a normal probability model. One model suggested by Pielou (1969) is a mixture of Poisson probability functions. It is assumed that female perch prefer to deposit egg-masses on coarse gravel substrates which are not too steep and are much less likely to spawn on steep slopes or a flat,

TABLE 3. Corrected counts (see text) of the numbers of yellow perch egg-masses observed along isobathic transects, 1978–86, Lochaber Lake, N.S.

Site/year	Transect depth (m)					Transect depth (m)					Transect depth (m)				
	4	6	8	10	12	4	6	8	10	12	4	6	8	10	12
	1978					*1979*					*1980*				
1	8	18	43	42	10	0	22	60	58	20	7	14	78	104	20
2	0	3	5	3	—	0	0	5	2	—	0	2	5	2	—
3	0	0	0	0	—	0	0	0	1	—	0	0	0	0	—
4	0	3	4	9	—	2	9	25	20	—	1	14	30	15	—
5	6	11	31	21	—	3	13	25	17	—	—	26	26	15	16
6	1	7	7	8	—	1	3	10	9	—	0	14	9	11	—
7	0	0	0	0	—	0	0	3	5	—	0	1	4	5	—
8	3	0	2	3	—	0	0	2	10	—	0	1	5	4	—
9	0	0	0	—	—	1	0	4	10	—	0	4	1	1	—
10	0	0	0	1	—	0	0	9	7	—	0	0	3	7	—
11	0	2	1	10	—	0	0	9	8	—	—	2	0	8	7
12	—	—	—	—	—	0	0	0	5	—	0	1	1	2	—
13	—	—	—	—	—	—	—	—	—	—	0	0	2	2	—
14	—	—	—	—	—	—	—	—	—	—	1	1	7	3	—
	1981					*1982*					*1983*				
1	3	6	18	60	25	0	5	40	73	30	—	1	31	50	32
2	0	0	4	2	—	0	1	5	4	—	—	0	2	0	1
3	0	0	0	1	—	0	0	0	0	—	—	—	—	—	—
4	0	0	0	1	—	—	4	7	38	4	—	1	18	25	9
5	6	13	21	21	8	1	10	5	17	15	—	9	13	7	11
6	—	0	3	3	7	0	1	4	3	—	—	0	3	21	11
7	0	0	1	0	—	0	1	0	3	—	—	—	—	—	—
8	0	1	0	2	—	0	1	1	4	13	—	3	3	16	5
9	1	1	0	0	—	0	0	1	4	—	—	0	4	7	2
10	—	0	0	1	15	—	0	4	11	11	—	0	3	13	5
11	0	0	2	9	—	0	1	12	10	—	—	0	2	7	5
12	0	0	0	2	—	0	0	0	3	5	—	—	—	—	—
13	—	0	0	0	2	1	0	3	10	—	—	—	—	—	—
14	0	0	0	1	—	0	3	5	7	—	—	0	3	4	8
15	0	0	0	1	—	0	0	0	4	—	—	—	—	—	—
16	—	0	3	7	7	—	0	2	4	5	—	0	1	9	3
17	—	—	—	—	—	—	—	—	—	—	—	0	2	19	5
18	—	—	—	—	—	—	—	—	—	—	—	1	5	3	4
	1984					*1985*					*1986*				
1	—	1	18	8	21	0	8	25	36	11	1	12	19	15	12
2	—	0	4	1	2	0	1	1	1	—	0	0	1	2	—
3	—	0	1	0	0	0	0	0	1	0	0	0	2	0	—
4	—	3	6	5	3	1	4	12	19	7	—	3	6	3	2
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	1	2	4	7	0	4	1	5	4	—	5	3	7	6
7	—	0	0	1	3	0	1	1	5	7	—	0	1	1	3
8	—	0	1	1	0	0	4	3	3	2	—	1	6	1	6
9	0	0	0	0	—	0	5	3	1	0	1	0	3	3	—
10	—	0	1	0	0	0	0	2	10	5	—	0	0	1	6
11	—	3	5	5	0	1	2	5	4	1	—	1	0	1	3
12	—	0	0	0	0	0	0	2	0	—	—	0	1	2	1
13	—	1	2	1	1	0	2	4	4	—	0	0	4	2	—
14	—	0	1	2	3	0	1	2	0	—	0	1	0	3	—
15	—	0	0	0	0	0	0	1	2	2	—	0	0	1	0
16	—	0	2	2	2	0	0	4	3	3	—	0	0	0	1
17	—	0	4	4	0	0	0	0	1	1	—	1	0	0	0
18	—	0	0	1	0	0	0	1	7	1	0	0	1	2	—

TABLE 4. Values of the parameters for the mixture of Poissons for all years and depths (see text).

Depth (m)	Parameter	Year								
		1978	1979	1980	1981	1982	1983	1984	1985	1986
4	$p[1]$	0.66	0.42	0.87	0.71	0	—	—	0	0
	$p[2]$	0.34	0.58	0.13	0.29	1.00	—	—	1.00	1.00
	$t[1]$	0.12	0	0.24	0	0	—	—	0	0
	$t[2]$	5.21	1.49	5.52	3.58	0.19	—	—	0.12	0.52
6	$p[1]$	0.75	0.79	0.75	0.88	0.83	0.87	0.51	0.60	0.89
	$p[2]$	0.25	0.21	0.25	0.12	0.17	0.13	0.49	0.40	0.11
	$t[1]$	1.11	0.71	1.78	0.35	0.85	0.60	0	0.53	0.73
	$t[2]$	13.6	16.8	19.0	9.99	7.07	6.91	1.38	4.35	10.0
8	$p[1]$	0.81	0.84	0.88	0.84	0.92	0.84	0.89	0.90	0.91
	$p[2]$	0.19	0.16	0.12	0.16	0.08	0.16	0.11	0.10	0.09
	$t[1]$	2.06	5.99	5.52	0.94	3.13	3.97	1.77	2.38	1.84
	$t[2]$	36.9	49.8	67.9	17.4	36.2	25.7	14.6	20.7	16.0
10	$p[1]$	0.81	0.88	0.92	0.91	0.89	0.82	0.60	0.89	0.91
	$p[2]$	0.19	0.12	0.08	0.09	0.11	0.18	0.40	0.11	0.09
	$t[1]$	4.52	7.96	5.82	2.84	6.52	8.68	0.95	3.37	2.03
	$t[2]$	35.4	51.9	100.0	54.0	62.8	40.1	4.81	30.2	12.6
12	$p[1]$	—	—	0.11	0.69	0.75	0.89	0.90	0.66	0.63
	$p[2]$	—	—	0.89	0.31	0.25	0.11	0.10	0.34	0.37
	$t[1]$	—	—	3.77	6.52	7.73	5.57	1.15	1.85	1.74
	$t[2]$	—	—	16.0	21.2	25.1	28.1	17.8	7.37	7.89

TABLE 5. Chi-square values for the egg-mass distribution models reported in Table 4.

Depth (m)	Year								
	1978	1979	1980	1981	1982	1983	1984	1985	1986
4	0.063	0.54	0.23	0.048	0.011	—	—	0.0034	0.43
6	0.0021	0.00032	0.010	0.20	0.14	0.50	0.39	0.028	0.61
8	0.0072	0.0059	0.00001	0.010	0.29	0.018	0.15	0.00023	0.0035
10	0.19	0.0060	0.011	0.027	0.054	0.030	0.0015	0.016	0.69
12	—	—	0.0063	0.00004	0.051	0.000	0.15	0.0049	0.0085

heavily silted bottom. Thus, the bottom is divided up into areas where female perch (1) rarely deposit egg-masses and (2) frequently deposit egg-masses. Given that a female perch deposits an egg-mass in one of these types of areas, it is assumed that the location within the area type is randomly selected, although it does depend on the depth. If X is the number of egg-masses counted in a 200-m^2 swath, the probability that n egg-masses will be counted is given by the formula

$$P(X = n) = p[1]\text{Prob}(n;t[1]) + p[2]\text{Prob}(n;t[2])$$

where $p[1] + p[2] = 1$, $p[1] > 0$, $p[2] > 0$, and $\text{Prob}(n;t) = t^n\exp(-t)/n!$ is the usual Poisson probability formula. The proportion of the bottom where female perch rarely deposit egg-masses is $p[1]$ and the mean density of egg-masses on this type of bottom is $t[1]$. Similarly, $p[2]$ is the proportion of bottom where female perch frequently deposit egg-masses, and $t[2]$ is the mean density of egg-masses on this bottom type.

The method of moments (see Appendix) provides a method for approximately computing the parameters $p[1]$, $p[2]$, $t[1]$, and $t[2]$ for the mixture of Poissons (Table 4). The cases where the parameters $p[1]$ and $p[2]$ are respectively 0 and 1.00 are cases where the variances were approximately the same as the mean (years 1982, 1985, and 1986 at the depth of 4 m). The near equality of the mean and variance is an indication that one Poisson will satisfactorily fit the data (cf. Pielou 1974). The other unusual cases are when $t[1] = 0$ which occurs for the years 1979 and 1981 at the depth of 4 m and in 1984 at the depth of 6 m. In these two cases the procedures outlined in the Appendix produced unreasonable results (negative values for $t[1]$). The model assumed in these two cases was one where $t[1] = 0$. The parameters for this model can be computed from the first two moments for X (see Appendix).

The assumption that some areas are preferred by female perch for egg deposition while others are less preferred should be reflected in reasonably constant values for the proportions $p[1]$ and $p[2]$ over time, and the year-to-year fluctuations in the number of egg-masses and thus mature female perch should be explained by changes in the density parameters $t[1]$ and $t[2]$. Note that the proportions at the depths 8 and 10 m are relatively constant and that the fluctuations can be attributed to the reasonably large changes in the density parameters which are the mean number per 200 m^2 in the two different egg-laying regions. At other depths, the fact that there are relatively few locations with nonzero counts may mask this observation, although even the parameters for the 6-m depth seem to be relatively constant.

TABLE 6. Ninety percent confidence intervals for mean egg-mass counts and expected population estimates by year and depth.

		Estimated egg-mass distribution			Estimated population			
Year	Depth (m)	Lower limit	Mean count	Upper limit	Lower limit	Mean population	Upper limit	Probability
1978	4	0.64	1.87	3.36	676	1 991	3 575	0.91
	6	1.55	4.19	7.18	4 217	11 447	19 599	0.90
	8	2.09	8.74	16.09	2 446	10 226	18 825	0.90
	10	4.30	10.34	17.30	3 817	9 180	15 357	0.90
Total					11 156	32 844	57 356	
1979	4	0.33	0.87	1.42	354	920	1 505	0.92
	6	0.92	4.07	7.50	2 501	11 103	20 468	0.90
	8	5.83	12.93	21.42	6 824	15 128	25 055	0.90
	10	7.33	13.05	20.50	6 509	11 582	18 197	0.90
Total					16 188	38 733	65 225	
1980	4	0.17	0.91	1.92	177	969	2 037	0.90
	6	2.86	6.02	9.64	7 797	16 441	26 316	0.90
	8	5.14	12.75	22.71	6 016	14 914	26 574	0.90
	10	5.14	13.29	26.00	4 565	11 799	23 079	0.90
	12	9.00	14.65	19.00	7 139	11 623	15 071	0.90
Total					25 694	55 746	93 077	
1981	4	0.25	1.02	2.00	266	1 088	2 125	0.91
	6	0.31	1.53	3.00	852	4 163	8 187	0.90
	8	1.13	3.57	6.31	1 316	4 174	7 385	0.90
	10	2.50	7.35	13.56	2 219	6 525	12 039	0.90
	12	6.33	11.13	16.33	5 023	8 825	12 956	0.90
Total					9 676	24 775	42 692	
1982	4	0	0.18	0.38	0	191	408	0.97
	6	0.88	1.91	3.00	2 387	5 209	8 187	0.90
	8	2.75	5.93	10.06	3 217	6 936	11 772	0.90
	10	6.19	12.66	20.63	5 492	11 239	18 308	0.90
	12	7.29	12.09	17.43	5 779	9 594	13 825	0.90
Total					16 875	33 169	52 500	
1983	6	0.46	1.41	2.54	1 259	3 849	6 927	0.90
	8	3.92	7.36	11.54	4 589	8 611	13 499	0.90
	10	9.00	14.40	20.54	7 989	12 784	18 231	0.90
	12	5.08	8.14	11.85	4 027	6 457	9 397	0.90
Total					17 864	31 701	48 054	
1984	6	0.29	0.67	1.12	803	1 840	3 050	0.91
	8	1.59	3.12	5.00	1 858	3 649	5 849	0.91
	10	1.53	2.48	3.47	1 357	2 201	3 080	0.91
	12	1.00	2.85	5.19	793	2 261	4 115	0.90
Total					4 811	9 951	16 094	
1985	4	0	0.12	0.24	0	128	250	0.94
	6	1.18	2.06	3.00	3 210	5 616	8 181	0.90
	8	2.18	4.21	6.71	2 546	4 927	7 845	0.90
	10	3.18	6.33	10.00	2 819	5 622	8 876	0.90
	12	2.31	3.72	5.23	1 830	2 950	4 149	0.91
Total					10 405	19 243	29 301	
1986	4	0	0.52	1.00	0	552	1 062	0.97
	6	0.65	1.72	3.06	1 765	4 684	8 347	0.90
	8	1.65	3.15	5.12	1 926	3 691	5 987	0.90
	10	1.76	2.96	4.41	1 566	2 627	3 916	0.90
	12	2.27	4.01	5.73	1 802	3 181	4 543	0.90
Total					7 059	14 735	23 855	

The goodness-of-fit of these parameters may be tested using the likelihood ratio test (cf. Kendall and Stewart 1967; Mood et al. 1974; Bickel and Doksum 1977). The resulting values for each of the above models are given in Table 5. Under the assumptions that these values are chi-square with one degree of freedom, the null hypotheses would be accepted with a critical region less than 0.25 in 28 of the 41 cases and with a 0.10 critical region in 19 of the cases. In contrast, a similar calculation for the best fitting normal model shows that the null hypotheses would be rejected with a 0.25 critical region in all but one case and would be rejected in all cases with a 0.10 critical region.

Given the probability functions by year and depth, the mean egg-mass count for a randomly selected 200-m^2 portion of the bottom can be computed for that year and depth. The formula for this expected value is

$$E(X) = p[1]r[1] + p[2]r[2].$$

Multiplying this figure by the factor (area at that depth)/200, an estimate of the population for that year and depth can be obtained. It is also possible to obtain the probability distribution function for the expected egg-mass count computed above (see Appendix), and thus, confidence intervals for these means can be obtained. Since most gravid female perch do spawn (Clady 1976), the total number of egg-masses corresponds to the number of mature females. Table 6 gives the means, 90% confidence intervals for these means, the corresponding expected populations, and 90% confidence intervals.

Since not all depths were counted each year, it is necessary to correct the above yearly totals (Table 6) to obtain comparable figures for each year's total population. One way to make a correction is to compute the average proportion of the egg-masses at the depth of 4 m (missing for 1983 and 1984) for those years for which counts were made and the average proportion of egg-masses at the depth of 12 m (missing for 1978 and 1979). These proportions are 0.036 for 4 m and 0.328 for 12 m. Using these proportions, the corrected estimates for the number of spawning females were as follows:

Year	1978	1979	1980	1981	1982	1983	1984	1985	1986
Population	42 964	51 136	55 746	24 775	33 169	32 610	10 228	19 243	14 735

The equation best describing the fecundity of perch in relation to age is given by (GM-regression, Ricker 1973)

$$\log(\text{fecundity}) = 2.662 + 0.198(\text{age})$$

$$(\text{SE} = 0.187,\ r^2 = 0.643).$$

From trap collections in early June, less than 5% of 2-yr-old female perch were observed to be gravid and those greater than age 2 were gravid or had obviously spawned.

The average contribution to recruitment by each age-class can be determined from a stable age distribution for the female perch. Such a population is given for the age-classes $x = 3, 4, \ldots, k$ by

$$C\begin{bmatrix} R^{k-3} \\ p(3)R^{k-4} \\ p(3)p(4)R^{k-5} \\ \vdots \\ p(3)p(4)\ldots p(k-1) \end{bmatrix}$$

TABLE 7. Age distributions of catches of female yellow perch, 1978–85, Lochaber Lake, N.S.

Age (yr)	Year						
	1978	1979	1980	1981[a]	1982[a]	1984	1985
3	172	78	29	59	33	253	157
4	42	20	17	35	68	221	176
5	23	16	8	10	32	180	137
6	8	7	6	4	7	83	68
7	9	1	1	3	3	18	16
8	5	2	1	0	0	3	13
9	1	0	0	0	0	0	7
10	0	0	0	0	0	1	0

[a]Captures of females too small; data represent pooled male and female captures.

TABLE 8. Stable age distribution and percent contribution to recruitment for female yellow perch, Lochaber Lake, N.S.

Age (yr)	Percentage	
	Population	Recruitment
3	52.2	30.4
4	24.4	22.4
5	13.4	19.4
6	7.5	17.3
7	1.6	5.8
8	0.8	4.6
9	0	0.3

where $p(x)$ are the age-specific survival probabilities for female perch of age x, C is an arbitrary constant, and R is the dominant root of the characteristic equation

$$R^k - P(m(3)R^{k-1} + p(3)(m(4)R^{k-2} + p(4)(\ldots(m\ (k-1)R + p(k-1)m(k))\ldots))) = 0$$

of the Leslie matrix for the female perch population. The parameter P is given by $P = p(0)p(1)p(2)$ and $m(x)$, $x = 3, 4, \ldots, k$ are the age-specific fecundities (cf. Aalto and Newsome (1980) for a more complete discussion).

The age specific probabilities ($p(x)$ and the parameter P can be determined by the method of least squares from estimates of total number of female perch in each age-class over a number of consecutive years. In turn, these estimates can be determined from the age samples collected during spawning times (see Table 7) and the population estimates computed above. If we restrict our attention to $k = 9$ age-classes, then the least squares estimates for $p(x)$ and P are as follows:

x	3	4	5	6	7	8
$p(x)$	0.428	0.502	0.515	0.193	0.461	0.004

and $P = 1.29 \times 10^{-4}$. The dominant root of the characteristic equation is $R = 0.915$. This yields Table 8 which gives the percentage of mature females in a stable age distribution and the corresponding percentage contribution that each age-class makes to recruitment for that year.

Multivariate regression analysis relating annual population estimates and a number of meteorological variables (all except variable 5) describing conditions 3 yr previous showed no significant relationships. The analysis did reveal a significant

relationship between estimates of female abundance at times t and $(t - 3)$. This is not a surprising relationship but its strength ($r^2 = 0.96, p = 0.002$) supports our observation that the majority of females attain maturity at age 3 and suggests that first-time spawners contribute significantly to annual recruitment. This observation is consistent with the stable age calculations made above.

Our study indicates the occurrence of large changes in survival rates during the period from embryo to mature female (3 yr old). More speculatively, it can be hypothesized that the between-year variability in the mortality rate beyond the first year is low. Thus the large fluctuations in year-class strengths and the population of perch in Lochaber Lake and other lakes would be due to substantial variations in the annual survival rates during the first year. These observations are consistent with Clady's (1976) results which showed that the survival rate during the life stages embryo to fry of 8 mm length varied from 1.6 to 18.4% for Oneida Lake perch. Thus the survival rate over this brief period (3–4 wk) varies by an order of magnitude.

An examination of the life cycle of perch and other organisms provides reasons for the above events. During the egg to larval stage, the most dramatic changes in structure and size occur. Thus, during this part of the life cycle there is the greatest possibility for variations in development which may lead ultimately to mortality or reduced chances of survival. Additionally, during this part of the life cycle, the organism has little or no mobility, and thus cannot respond to changes in the local physical and chemical environment such as temperature, currents, oxygen concentration, etc. By contrast, juvenile and adult fish can and do move in response to stimuli such as availability of food and changes in light, dissolved oxygen, or temperature.

In our own observations in Lochaber Lake, we have observed large temperature (Celsius) changes at a depth of 10 m (as much as 7° in 15 min). Further, results of a preliminary study (G. E. Newsome, unpublished) show that perch embryos which have been subjected to frequent thermal shocks of as little as 2° are more likely to result in hatch fry exhibiting abnormal gill and jaw development, smaller body size, and smaller pectoral fins that is normal for embryos maintained at a constant or slightly rising temperature. Severe deformities will lead to immediate death, while less severe deformities may have a significant effect on survival of the perch at other critical life stages during the first year, and perhaps even during later life stages. This mechanism can explain how the year-class strengths may be determined during egg and larval stages in contrast with Nielsen's (1980) assertion. For example, poor fin development may lead to greater susceptibility to predation.

Wind and water temperature are frequently cited as factors affecting the survival of young-of-the-year perch (Jobes 1952; Pycha and Smith 1955; Clady and Hutchinson 1975; Clady 1976), and efforts to correlate wind velocities with perch year-class strengths are often made. We speculate that the above-described temperature variations are due to wind-driven internal seiches. We plan to study the relationship between winds and the dynamics of internal seiches which we think will explain much of the variation in the survival of the early life stages of perch. We note, however, that each lake where wind-driven seiches may have a large effect on the population will have its own dynamics, and thus will have to be analyzed separately.

Clady (1976) observed substantial correlations between survival of early life stages of perch and wind velocities without regard to direction. We found no significant relationship between total wind velocities and annual variations in perch numbers in Lochaber Lake. However, correlations between directional wind velocity vectors for the month of June (1978–84) and an index of relative change in female abundance (estimated abundance at time (t) divided by estimated abundance at time $(t - 3)$) suggest that winds from a northerly direction may play an important role ($r^2 = 0.765, p = 0.07$) in relation to embryonic or early larval survival.

The discrepancy between our initial observations and those of Clady (1976) concerning wind effects may be due to differences in lake orientation and morphology. In Oneida Lake (Clady 1976), winds from most directions may generate significant internal circulation patterns whereas in Lochaber Lake, which is much narrower and deeper, only winds from specific directions would be capable of generating internal seiches. Winds from almost any direction could produce "sloshing" internal seiches in Oneida Lake, but only winds from a northeasterly or southwesterly direction would be effective in generating "rocking" internal seiches in Lochaber Lake. More detailed analyses of our data and additional data may shed light on the influence of this environmental feature in generating annual fluctuations in perch abundance.

Clady (1976) suggested that no major mortality factor but rather a complex interaction of many minor factors is responsible for annual variations in perch numbers. Our hypothesis would attribute the mortality due to these minor factors to potentially catastrophic events during embryonic stages, particularly wind-mediated thermal variations.

Thus far, we have offered one explanation for the large annual variations in year-class strengths. However, there is another contributing factor which can significantly affect these variations: the population may consist of a number of subpopulations which vary independently. Coincidence of peaks in abundance between subpopulations may lead to large populations one year, while other years may exhibit relatively reduced numbers.

Independent subpopulations can arise if perch exhibit demic structure. A number of authors have offered evidence that perch populations exhibit demic behavior based on morphological differences (Chikova 1973; Fortin and Fournier 1977; Leary and Booke 1982), tag returns (Rodeheffer 1941; Mansueti 1960; Muncy 1962; Williams 1965; Fortin and Magnin 1972; Kelso and Ward 1977; Clady 1977; Thorpe 1977), and spawning site selection (Dorr 1982). Our results do not provide direct support for demic behavior by the Lochaber perch population, but do provide support for the fact that year-to-year spawning occurs at specific sites on the bottom of Lochaber Lake. A precondition for demic behaviour by a perch population is that there are preferred sites where female perch may return each year to spawn. We expect that further analysis of our data will provide support for the demic hypothesis.

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Appendix

Determination of Parameters

Let X be a random variable taking the values $0, 1, 2, \ldots$, let $P(X = n)$, $n = 0, 1, 2, \ldots$, be the probability function for X,

and let $X_1, X_2, \ldots, X_q$ be a random sample from the population having $P(X = n)$ as its probability function. The kth factorial moment of X is given by

$$E(X^{(k)}) = \sum_{n=0}^{\infty} n(n-1)\ldots(n-k+1)P(X=n).$$

The kth sample factorial moment is

$$m_{(k)} = \frac{1}{q}\sum_{j=1}^{q} X_j(X_j-1)\ldots(X_j-k+1).$$

If

$$P(X=n) = \sum_{i=1}^{2} p[i]\frac{(t[i]^n \exp(-t[i])}{n!}$$

is a mixture of Poisson probability functions, then the factorial moment generating function (Mood et al. 1974) for X is

$$E(s^X) = \sum_{i=1}^{2} p[i]\exp(t[i](s-1))$$

and thus the kth factorial moment of X in terms of $p[i]$ and $t[i]$ is

$$E(X^{(k)}) = \frac{d^k}{ds^k}E(s^X)\Big|_{s=1} = \sum_{i=1}^{2} p[i](t[i])^k.$$

Thus the parameters $p[1]$, $p[2]$, $t[1]$, and $t[2]$ may be approximately computed by the method of moments (Bickel and Doksum 1977; Mood et al. 1974; Kendall and Stuart 1958). This method yields the following set of equations

$$\sum_{i=1}^{2} p[i](t[i])^k = m_{(k)}, \qquad k = 0, 1, 2, 3.$$

(Note: $m_{(0)} = 1$.)

This set of four nonlinear linear equations can be explicitly solved (using MAPLE, a symbolic manipulation package distributed by WATCOM) to obtain the following formulae for the parameters in terms of the sample moments $m_{(1)}$, $m_{(2)}$, and $m_{(3)}$:

$$t[2] = (m_{(1)}m_{(2)} - m_{(3)} \pm D^{1/2})/2(m_{(1)}^2 - m_{(2)})$$

where

$$D = m_{(3)}^2 - 6m_{(1)}m_{(2)}m_{(3)} - 3m_{(1)}^2m_{(2)}^2 + 4m_{(2)}^3 + 4m_{(3)}m_{(1)}^3$$

$$t[1] = (m_{(2)} - m_{(1)}t[2])/(m_{(1)} - t[2])$$

$$p[2] = (m_{(1)} - t[1])/(t[2] - t[1])$$

$$p[1] = 1 - p[1].$$

Note that if negative values are obtained for either $t[1]$ or $t[2]$, then one can set $t[1] = 0$ to obtain the following set of three equations for $p[1]$, $p[2]$, and $t[2]$,

$$p[1] + p[2] = 1$$

$$p[2]t[2] = m_{(1)}$$

$$p[2](t[2])^2 = m_{(2)},$$

for a mixture of Poisson's, with one of the parameters ($t[1]$) equal to zero.

The method of moments leads to methods for obtaining the parameters which are easy to calculate, and in the case of large samples the approximations for the parameters will be close to the parameters being estimated (Bickel and Doksum 1977). This method generalizes to mixtures of three or more Poissons, but the resulting set of equations for the parameters must be solved by a numerical method such as Newton's method.

Probability Function for the Mean of a Mixture of Poissons

If

$$P(X=n) = \sum_{i=1}^{h} p[i]\frac{(t[i])^n \exp(-t[i])}{n!}$$

is a mixture of Poisson probability functions, then the expected value of X is

$$E(X) = \sum_{i=1}^{h} p[i]t[i].$$

If $X_1, X_2, \ldots, X_q$ is a random sample, then the moment generating function (Mood et al. 1974) for

$$Y = \sum_{i=1}^{q} X_i$$

is

$$E(s^Y) = (E(s^X))^q.$$

For $h = 2$, this yields

$$E(s^Y) = (p[1]\exp(t[1](s-1)) + p[2]\exp(t[2](s-1)))^q$$

$$= \sum_{j=0}^{q}\binom{q}{j}(p[1])^j(p[2])^{q-j}\exp((jt[1] + (q-j)t[2])(s-1))$$

which can be recognized as the moment generating function for a mixture of q-Poisson distributions functions with mixture parameters

$$\binom{q}{j}(p[1])^j(p[2])^{q-j}$$

and Poisson parameters

$$r[j] = jt[1] + (q-j)t[2].$$

This mixture of q-Poissons is the distribution function for Y, and thus

$$P(E(X) = n/q) = \sum_{j=0}^{q}\binom{q}{j}(p[1])^j(p[2])^{q-j} \times \frac{(r(j])^n \exp(-r[j])}{n!}.$$

If $h > 2$, a more complicated expression may be derived using the multinomial expansion formula. A similar, although more complicated result will be obtained.

Food and Habitat Utilization by Juvenile Salmonids in the Campbell River Estuary

J. Stevenson Macdonald, I. K. Birtwell, and G. M. Kruzynski

Department of Fisheries and Oceans, Fisheries Research Branch, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6

Macdonald, J. S., I. K. Birtwell, and G. M. Kruzynski. 1987. Food and habitat utilization by juvenile salmonids in the Campbell River estuary. Can. J. Fish. Aquat. Sci. 44: 1233–1246.

Salmonid behaviour and abundance in several microhabitats within the Campbell River estuary was observed monthly, from May to July, by divers using snorkels and face masks. Concurrent vertical profiles of physical and biological parameters at each microhabitat were taken to characterize habitats frequented by the fish. Data were collected at high and low tide to record behavioural reactions to changes in water velocity, salinity, and temperature associated with tidal height and salt wedge intrusion. Samples of plankton collected at each microhabitat were compared with stomach contents of salmonids caught nearby to determine if interspecific differences in diet could be correlated with differences in the habitats they occupied. Fish occurred in loose assemblages, aligned with the current, feeding near estuarine banks. As water velocities increased with the ebbing tide, the fish concentrated in a shear region near the mouth of a slough and behind large rocks and submerged vegetation. At both high and low tide, larger coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) (usually hatchery reared) were in deeper, frequently more saline water and further from shore than the smaller conspecifics. Hatchery chinook, however, were also seen in sloughs where water velocity was low. Marine influence as reflected in plankton composition and salmonid diet was greater in the outer estuary and in the deep salt water that intrudes the inner regions of the estuary. Differences in the habitats occupied by the fish were reflected in differences in their diets.

De mai à juillet, des plongeurs munis de tubas et de masques ont observé mensuellement le comportement et l'abondance de salmonidés peuplant plusieurs microhabitats de l'estuaire de la rivière Campbell. Des profils verticaux simultanés de paramètres physiques et biologiques ont été notés comme caractéristiques des microhabitats fréquentés. Des données ont été recueillies à marée haute et à marée basse afin de déterminer les réactions comportementales aux variations du courant, de la salinité et de la température liées à la hauteur de la marée et à la remontée du coin salé. Des échantillons de plancton recueillis dans chaque microhabitat ont été comparés aux contenus stomacaux de salmonidés capturés dans les eaux avoisinantes afin de déterminer si des différences interspécifiques entre les régimes alimentaires peuvent être corrélées à des différences entre les habitats fréquentés. Les poissons étaient présents en groupements clairsemés disposés en ligne avec le courant et se nourrissaient près de bancs estuariens. Quand le courant augmentait avec le reflux de la marée, les poissons se regroupaient dans une zone de cisaillement près de l'embouchure d'un marais, et derrière de gros rochers et dans la végétation submergée. À marée haute et à marée basse, les gros saumons coho (*Oncorhynchus kisutch*) et quinnat (*Oncorhynchus tshawytscha*) (provenant généralement de piscifactures) fréquentaient des eaux plus profondes, souvent plus salées et à une plus grande distance du rivage, que leurs petits congénères. Toutefois, les saumons quinnat d'élevage ont aussi été observés dans des marais où le courant était faible. L'influence du milieu marin, qui est révélée par la composition du plancton et le régime alimentaire des salmonidés, était plus prononcée dans l'estuaire extérieur et dans les eaux salées profondes qui remontent vers les régions intérieures de l'estuaire. Les différences entre les habitats fréquentés par les poissons se sont traduites par des différences de régime alimentaire.

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The importance of estuaries as nursery zones for anadromous salmonids along the North Pacific coast has been well documented (Healey 1982; Myers and Horton 1982). During their estuarine residency, juvenile salmonids prey upon organisms of freshwater, estuarine, and marine origin (Healey 1980; Levy and Northcote 1981; Wolf et al. 1983). In addition to supplying food, estuaries may also provide sanctuaries from predators (McCabe et al. 1983) and are the brackish water habitats for physiological adaptation to marine conditions (Iwata 1981; Iwata and Komatsu 1984).

Food and space resource partitioning and the territorial behaviour of salmonids has been better documented in freshwater than during estuarine residence (e.g. Lister and Genoe 1970; Shirvell and Dungey 1983). In the estuary they encounter tidally influenced dynamic conditions quite different from those within river habitats (McInerney 1964). Most notably, during a flooding tide, a wedge of saline water intrudes into upper estuarine regions of most northern Pacific estuaries (e.g. Somass estuary, Tully 1949; Columbia estuary, Jay 1984; Campbell estuary, Piercey et al. 1985). Fish faced with changing

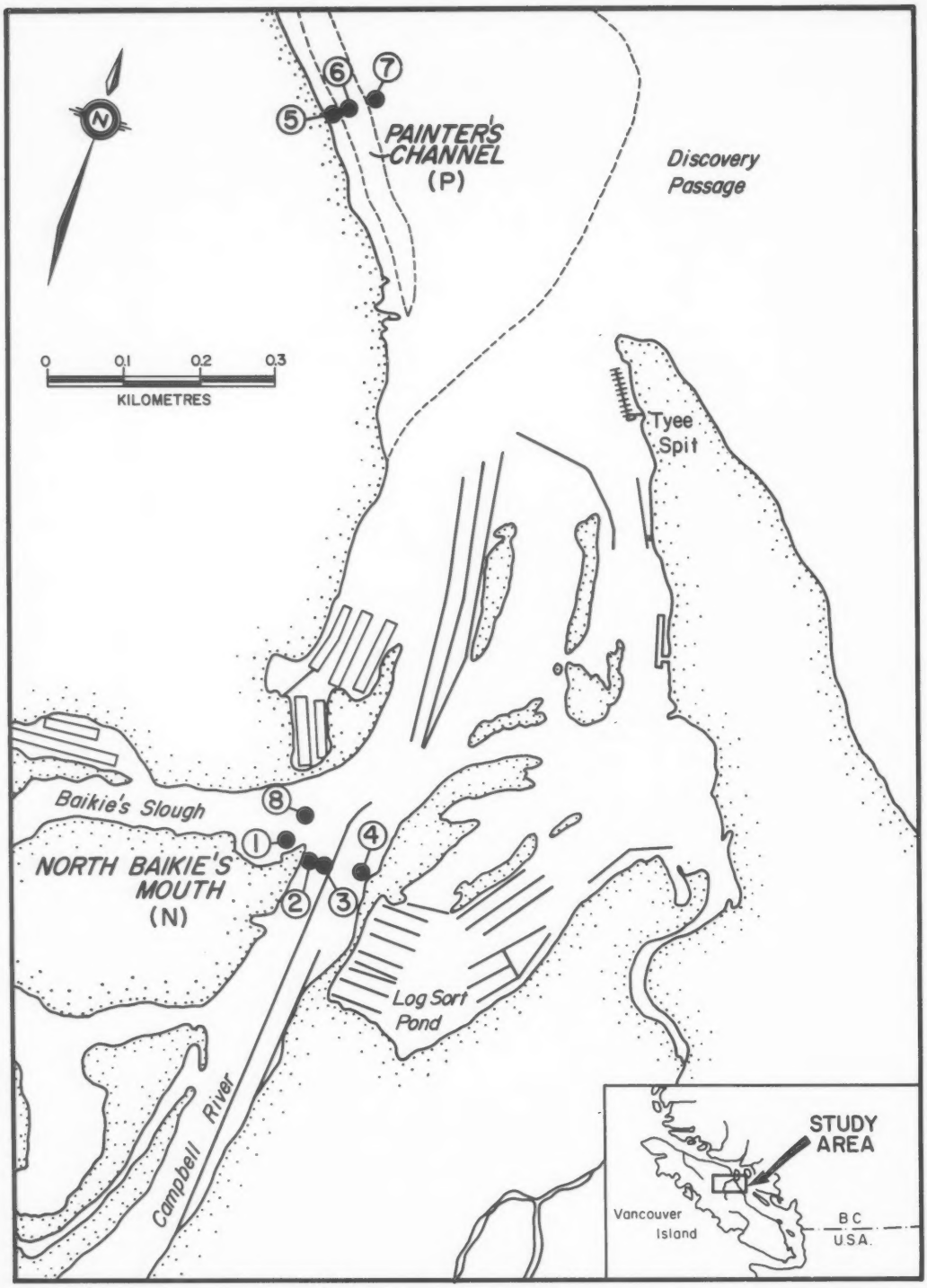

FIG. 1. Campbell River estuary showing the eight stations (microhabitats) at two regions (outer estuary: Painter's Channel; inner estuary: North Baikie's mouth). Descriptions of each station are given in Table 1. Beach seine collections of salmonids were made at Stations 1 and 7. Broken lines indicate the shoreline at times of maximum low tide. Solid lines in the estuary are log strings held in place by piles, or docks.

TABLE 1. Description of the eight study stations at two regions in the Campbell River estuary (Fig. 1). Estuary bottom profiles at each region are given in Fig. 2a and 2b. Depths were measured at a maximum high tide. Counts of fish (by divers) and plankton samples were made in the upper and lower water column, at high and low tide at each station (Table 2).

Region	Station	Description
N	1. North Baikie's refuge	Slough habitat; low currents; shelter from river current; 1 m deep; subtidal eelgrass beds (*Zostera* sp.)
N.	2. North Baikie's north side	On northwestern bank; exposed to river current; 1 m deep; undercut bank
N	3. North Baikie's middle	Midchannel; strong river current at low tide; salt wedge (2 m deep) present at high tide; 4 m deep
N	4. North Baikie's south side	On southeast bank; exposed to river current; 1 m deep; bank composed of vertical riprap
P	5. Painter's Channel west side	On western edge of channel; 3 m deep; shallow sloping large rock cobble bottom with *Enteromorpha* sp.
P	6. Painter's Channel middle	In midchannel; 4 m deep; kelp litter and eelgrass beds (*Zostera* sp.)
P	7. Painter's Channel east side	On eastern bank; 3 m deep; shallow sloping sand bottom with little vegetation
N	8. NBM side shear zone	In confluence of Campbell River and Baikie's slough; 1 m deep

food supplies and habitat may alter their patterns of resource use. For example, upon commencing downstream migration and during estuarine residence, salmon typically become less territorial and adopt a schooling behaviour (Keenleyside and Yamamoto 1962; Mason 1974; Gibson 1983).

This study had three objectives: (1) to characterize the microhabitats (Allee et al. 1949) occupied by salmon by defining physical features such as water velocity, salinity, and temperature, (2) to correlate gut contents of salmon captured with food presence in the habitats that they occupied (are there interspecific differences in diet, and if so, do these support our conclusions regarding habitat selection), and to investigate the influence of inter- and intraspecific aggression on microhabitat selection.

Study Area

The Campbell River estuary is located on the east coast of Vancouver Island, British Columbia, Canada (Fig. 1). Freshwater entering the estuary drains a basin of 1741 km^2. The predominant flows are from the Campbell and Quinsam rivers, which have a combined mean annual discharge of about 108 $m^3 \cdot s^{-1}$ (Bell and Thompson 1977). Flows in the lower reaches of the Campbell River are controlled and regulated through the John Hart power generating station which is located approximately 5 km upstream of the river mouth. Sediment transport into the estuary is therefore quite limited, and water clarity tends to be high.

The Campbell River discharges into the highly turbulent marine waters of Discovery Passage, thus confining the major influence of freshwater to the "inner estuary" behind Tyee Spit (Fig. 1). Seawater intrusion due to tidal action typically creates a vertically stratified estuary of approximately 73 ha at high tide (Brownlee et al. 1984). A variety of urban and industrial activities occur in the watershed (e.g. logging, mining, housing developments). In 1974 a fish hatchery was constructed 6 km upstream of the estuary on the Quinsam River. Coho salmon (*Oncorhynchus kisutch*, 3 863 312), pink salmon (*Oncorhynchus gorbuscha*, 6 947 445), rainbow trout (*Salmo gairdneri*, 25 400), and chinook salmon (*Oncorhynchus tshawytscha*, 1 411 758) were released from the facility during May and June 1984.

Eight sampling stations (Table 1; Fig. 1) in two contrasting regions of the Campbell River estuary were marked by buoyed groups of concrete blocks chained together to resist movement by strong water currents. Painter's Channel, proximal to Discovery Passage at the mouth of the estuary, always contains seawater but is also influenced by freshwater of the Campbell River at water levels 2 m above chart datum (maximum tidal height is approximately 4.5 m). North Baikie's mouth is located in the inner estuary, approximately 1 km upstream from Painter's Channel, and is therefore influenced to a greater extent by freshwater.

Materials and Methods

On three occasions (May 29 to June 1, June 25 to 27, and July 23 to 26, 1984), two divers, using snorkel equipment, counted juvenile salmonids (five replicates, 1-min observations) at each of the eight stations during high and low tide. Divers were held motionless on station by lines while fish were identified to species and their sizes were estimated. Because of variations in visibility, we restricted counts to a 3-m^3 area to one side of each diver. When water depth permitted, separate counts were made within and below 1 m of depth to facilitate correlations with physical and biological characteristics of the water column. A more detailed description of fish behaviour, including estimates of the exact position fish occupied within the water column, proximity to cover, and aspects of territoriality or feeding activities, was recorded following each 5-min counting session. An additional 25 h of underwater observations of salmon habitat at both inner and outer estuarine regions were made by each diver during the three sampling trips. During this time, habitats examined were those that were

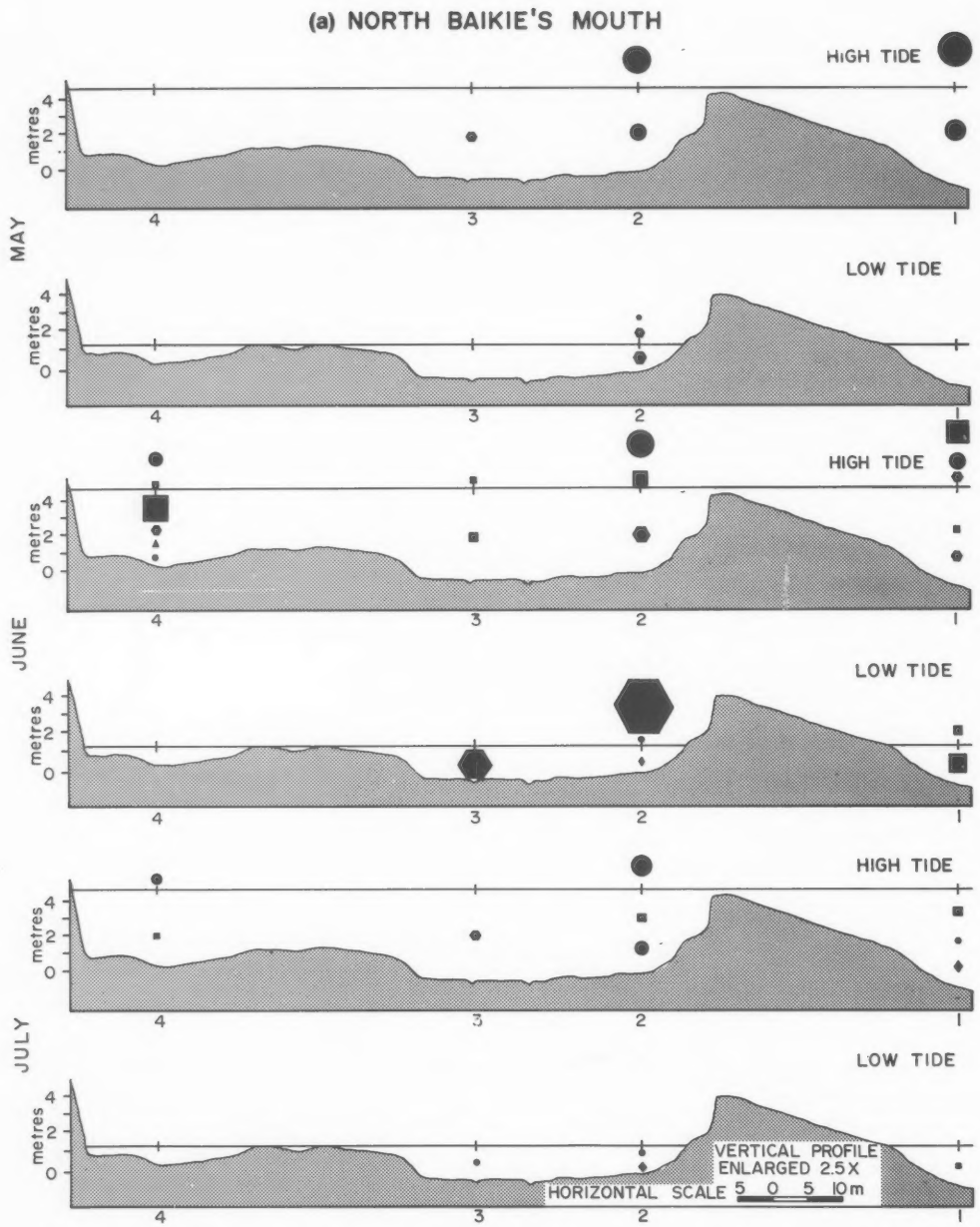

FIG. 2. Cross-sectional diagrams of the estuary at (a) North Baikie's mouth and (b) Painter's Channel. Horizontal lines indicate the water level at high and low tide during each of the three collection trips. Microhabitats are identified by station number (beneath each cross-sectional plot) with the exception of NSH (the shear zone, Station 8). The area of the symbols at each station is proportional to the mean number of fish observed at that site. Symbols above the horizontal water level line are numbers of fish observed in the surface 1 m of water; those below the line represent fish observed in water deeper than 1 m. ■, hatchery chinook; ●, wild chinook; ⬣, hatchery coho; ◆, cutthroat; ▲, steelhead.

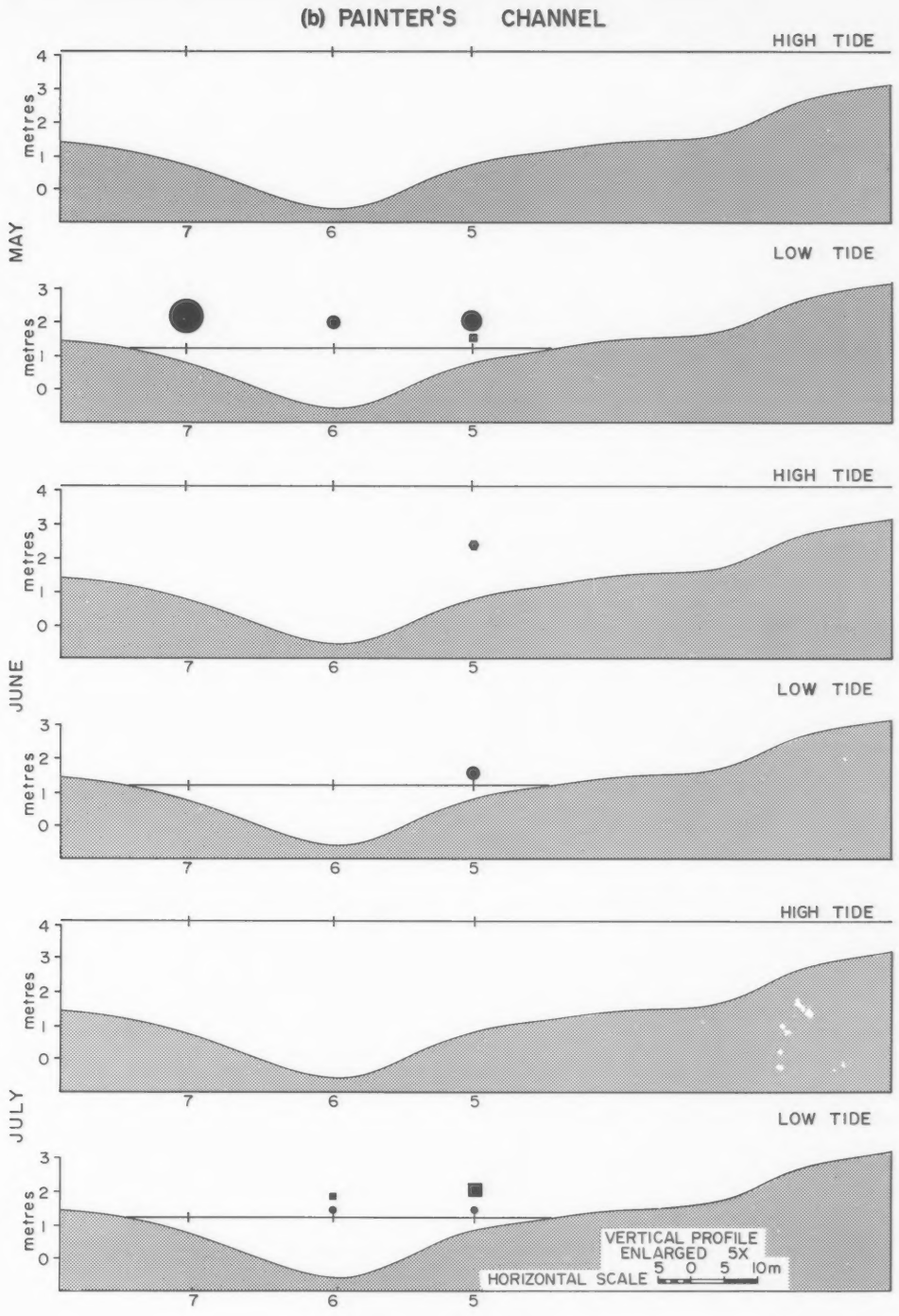

FIG. 2. (*Concluded*)

not visible to the divers from their observation stations (e.g. bank cover). Effort was made to minimize the use of motors to avoid influencing fish behaviour.

The greatest numbers of fish were observed during late June at North Baikie's mouth coincidental with large releases of coho and chinook salmon from the Quinsam River hatchery (Fig. 2a and 2b). Divers found it relatively easy to make underwater distinctions among the hatchery coho, hatchery

TABLE 2. Capture locations (microhabitat stations) and sample sizes of plankton and fish from inner and outer estuarine sites at low and high tide during the three collection trips. Fish were collected by beach seine from Stations 1 and 7. Mean fork lengths are presented in parentheses. Abbreviations as used in Fig. 4, 5, 6, and 7 and Tables 3 and 4.

Sample type/tide height (H or L)/location (N or P)	Stn.	Sample size May	June	July
Fish				
Chum high tide Baikie's (CMHN)	1	1 (104.0)	8 (71.6)	
Chum low tide Baikie's (CMLN)	1		3 (76.7)	
Chum low tide Painter's (CMLP)	7	4 (76.5)	14 (69.2)	
Coho high tide Baikie's (COHN)	1	10 (124.4)	18 (106.6)	
Coho low tide Baikie's (COLN)	1	11 (116.1)	10 (107.2)	7 (109.6)
Coho low tide Painter's (COLP)	7	16 (68.9)	1 (143.0)	
Wild chinook high tide Baikie's (WCHN)	1	14 (48.7)	17 (68.0)	
Wild chinook low tide Baikie's (WCLN)	1		20 (64.8)	8 (70.9)
Wild chinook low tide Painter's (WCLP)	7		11 (65.0)	12 (84.2)
Hatch chinook high tide Baikie's (HCHN)	1	19 (80.5)		
Hatch chinook low tide Baikie's (HCLN)	1	3 (77.0)		
Hatch chinook low tide Painter's (HCLP)	7	3 (79.7)		
Cutthroat low tide (CULN)	1	2 (143.5)		
Plankton				
Low tide slough surface (LNR)	1	4	4	4
High tide slough surface (HNR)	1	4	4	4
Low tide Baikie's shear surface (LNSH)	8			4
High tide Baikie's shear surface (HNSH)	8			4
Low tide Baikie's north surface (LNN)	2	4	4	
High tide Baikie's north surface (HNN)	2	4	4	
Low tide Baikie's midsurface (LNM)	3	3	4	4
High tide Baikie's midsurface (HNM)	3	4	4	4
Low tide Baikie's south surface (LNS)	4	4	4	4
High tide Baikie's south surface (HNS)	4	4	4	4
High tide Baikie's slough salt wedge (HNRS)	1	4	4	4
High tide Baikie's middle salt wedge (HNMS)	3	4	4	4
Low tide Painter's east surface (LPE)	7	4	4	4
High tide painter's east surface (HPE)	7	4	4	4
Low tide Painter's midsurface (LPM)	6	4	4	4
High tide painter's midsurface (HPM)	6	4	4	4
High tide Painter's west surface (HPW)	5	4	4	4
High tide Painter's middle salt wedge (HPMS)	5	4	4	4

chinook, and wild chinook salmon. Hatchery coho (1+) and chinook were generally larger than wild fish (Table 2) and often had their adipose fin removed. Parr mark shape and body colouration also aided in identification. Chum (*Oncorhynchus keta*), wild chinook, and coho (0+) were more difficult to distinguish, since they were much smaller than the hatchery fish and tended to aggregate. Because of these difficulties only those fish that divers could clearly identify as wild chinook were tabulated (Fig. 2a and 2b).

Temperature (degrees Celsius), dissolved oxygen (milligrams per litre), light energy (micro Einsteins per second per square metre), water current velocity (centimetres per second), and salinity (parts per thousand) were recorded at 0.33-m intervals in the water column at each of the eight stations immediately after observations of fish by divers. Procedures and data are presented in Piercey et al. (1985). Total water depth was also recorded. Plankton samples were collected ($n = 4$) at 0.3 and 1.3 m at most of the sampling stations immediately following measurement of the physical variables (Table 2). Two high-speed Miller nets (11.5 cm in diameter, 350-μm mesh size), mounted 40 cm apart and parallel to each other, were used on a weighted or buoyant frame for the collection of subsurface and surface samples, respectively. The nets were towed by boat or manually in shallow water for 1–2 min at approximately 6 $cm \cdot s^{-1}$. A flowmeter attached to the samplers permitted numbers of plankton to be related to a standardized water volume (cubic metres). Plankton were preserved in 10% formalin and then identified and enumerated in the laboratory.

Immediately after taking the plankton samples, fish were collected from each region (Stations 1 and 7; Fig. 1) using a 13.8 × 2.0 m (1-cm stretched mesh) beach seine deployed from a boat. The beach seine operated in the top 1.5–2 m of the water column and therefore it was impossible to separate fish collected from upper and lower water column locations. Random subsamples ($n < 20$) of juvenile hatchery-reared coho and chinook and wild chum and chinook salmon were collected and preserved in 10% formalin (Table 2). Determinations of fork lengths, wet weights, and stomach contents were made in the laboratory.

Prey items consumed by each fish species were compared with plankton collected from stations in the same region using Schoener's (1970) similarity index:

$$S = 100(1 - 0.5 \Sigma |P_{xi} - P_{yi}|)$$

where P_{xi} is the proportion of item i in the diet of species x and

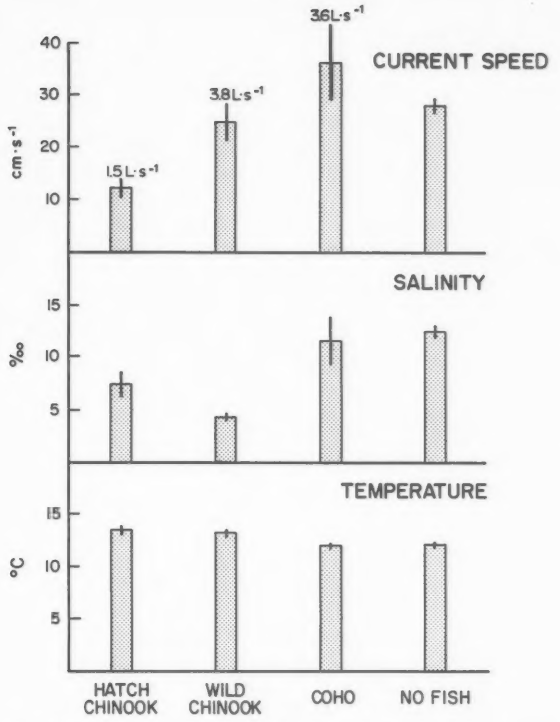

FIG. 3. Mean current speed, salinity, and temperature in microhabitats where hatchery and wild chinook salmon, coho salmon, and no fish were observed (n = 97). These were the three physical parameters that differed significantly among the microhabitats occupied by these fish (discriminant analysis, $P < 0.05$). Vertical bars represent 2 SE of the mean. Mean current speeds occupied by each species of fish, as expressed in body lengths (L) per second, are also presented above each bar.

P_{yi} is the proportion of item i in the plankton samples. Values range from 0 (no similarity between diet and available plankton) to 100 (identical).

A data matrix consisting of measurements of the physical variables (temperature, dissolved oxygen, water depth, light energy, water velocity, and conductivity) from depths of 0.33, 1.33, 2.33, and 3.33 m in the water column, with coincident estimates of fish and potential prey abundance at each depth, was constructed using the data gathered from both estuarine regions during the study. In creating the matrix, we chose from the physical variable measurements that had been made at 0.33-m intervals, the measurements from the depths at which the fish were most frequently seen. Potential prey abundance estimates were not available at 2.33 and 3.33 m. Discriminant functions analysis (Orloci 1978; Jennrich and Sampson 1979) was used to compare values of the physical variables and plankton abundance that typified habitats occupied by hatchery chinook, hatchery coho, and wild chinook salmon and measurements of conditions where no fish were observed at all. Variables that differed significantly ($P < 0.05$) among fish habitat types were identified.

The significance of differences in the diets of each fish species captured on the same date at Painter's Channel and North Baikie's mouth at low and high tide was also compared using discriminant functions analysis. Variables (prey types) that loaded heavily on the canonical axes (and therefore were consumed in differing amounts among the fish species collected at each tide height in each region; $P < 0.05$) were tabulated. Data were transformed by ln (X + 1) before analysis to remove dependence of the variance on the mean (Green 1979). Plankton sample data were analyzed by the same method. That is, plankton composition in samples from each depth at each station in each region at both high and low tide was compared and plankton species that differed in abundance among groups were tabulated.

Results

Water velocity, temperature, and salinity were the variables that were most important in characterizing differences among microhabitats occupied by the salmonids (discriminant analysis, $P < 0.05$; Fig. 3). Coho salmon were observed in regions of higher salinities and water velocities than chinook salmon. Hatchery chinook salmon were in lower velocity waters than all the other salmonids. With the increase in water velocities associated with low tide (Fig. 4), fewer hatchery and wild chinook salmon were seen at Stations 2–4 at North Baikie's mouth (Fig. 2a). Coho salmon, however, were still seen at these sites at low tide. Some of the hatchery chinook may have been displaced to slough habitats where water velocity was low and the water was warmer (e.g. Station 1; Fig. 2a, 3, and 4). Wild chinook were not seen during low tide in the slough or at other NBM stations, but large numbers of them moved into the shallow marginal areas adjacent to the current near Station 2. Fish in the cover provided by marginal areas were generally out of sight of the divers during the fish counting period (Fig. 2) but were seen frequently at low tide during the times divers explored the estuary. Large numbers of wild chinook were counted in the outer estuary (e.g. Painter's Channel; Fig. 2b) where water velocity was lower at low tide than at high tide (Fig. 4). Concentrations of both hatchery and wild salmonids were seen (but not counted) at Station 8. Unlike other stations at North Baikie's mouth, there was relatively little vertical salinity, temperature, or flow rate stratification at this station, indicating it to be a region where incoming marine water and outgoing river water mixed (Fig. 4).

Larger fish, namely cutthroat trout (*Salmo clarki*) and hatchery coho and chinook salmon, seen at both inner and outer estuarine regions, were observed more often further from shore in the deeper (>1 m) more saline water of the salt wedge (30‰) than the wild chinook (Fig. 2, 3, and 4). For example, at high and low tide, hatchery coho and chinook salmon were observed frequently near the bottom of the channel at Station 3 (Fig. 2a). Station 3 was further from shore and deeper than all other stations and had among the highest salinities at high tide and the highest current velocities at low tide.

At most stations, fish held a constant position aligned with the current in loose aggregations and rarely showed agonistic behaviour (charging, chasing, or displays; Keenleyside and Yamamoto 1962). Occasional horizontal dashes after food or to the surface, presumably to feed or to adjust buoyancy, were followed by rapid swimming to return to approximately the same location the fish had left. When water velocity was low (e.g. in slough habitat, Station 1), fishes did not orient in any particular direction and were seen singly or in smaller aggregations than those seen in stronger currents. Therefore, it was difficult to identify interspecific differences in behaviour in the slough. However, proportionally more fish of all species were

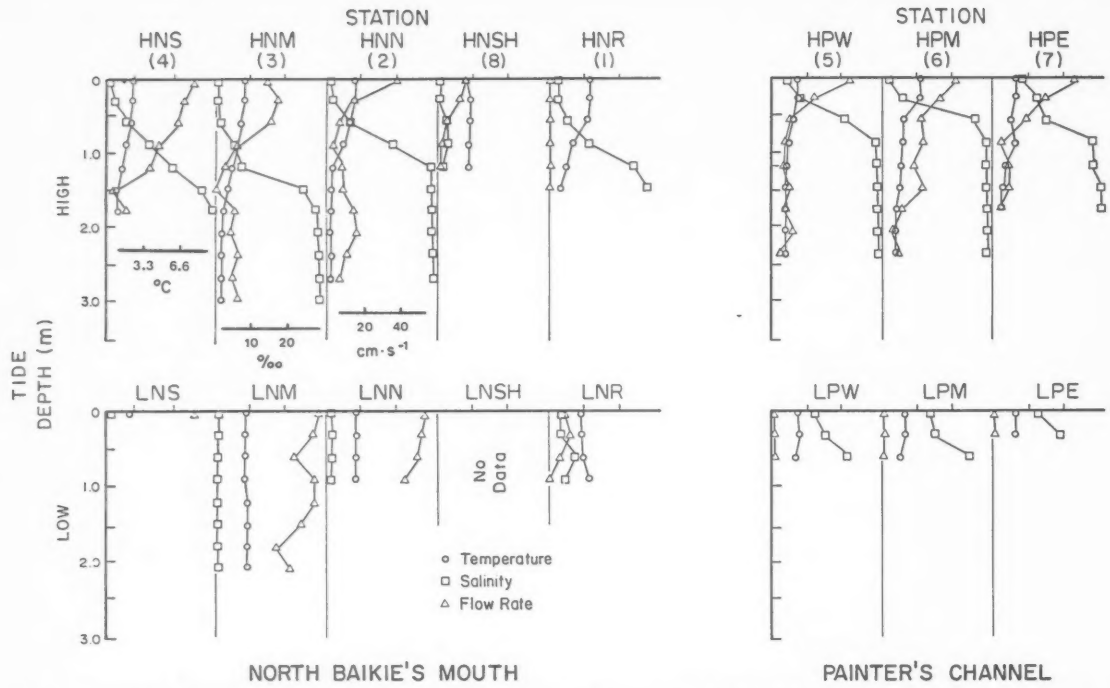

FIG. 4. Temperature, salinity, and flow rate profiles at each of the eight stations taken at high and low tide. Station numbers and descriptions correspond to those presented in Fig. 1 and Table 1. Station abbreviations are given in Table 2. These profiles were observed during the second collection trip (late June). They are representative of profiles observed during the first and third collection trips.

seen near the bottom of the slough during the July trip than during other trips (Fig. 2).

Fish, particularly those that were observed frequently in the upper freshwater layer (e.g. wild chum and chinook), underwent a dietary shift from food of freshwater origin at the inner estuary location (e.g. *Bosmina* sp. and insects; Table 3) to food of estuarine or marine origin at the outer estuary (e.g. *Neocalanus* sp., *Cumella vulgaris*; Table 3) (discriminant function I, $P < 0.05$; Fig. 5). This shift, to a large degree, is a reflection of the increased amounts of marine and estuarine plankton present at the outer estuary region (e.g. cumaceans, calanoids, and harpacticoids; Table 4) (discriminant function I, $P < 0.05$; Fig. 6). Marine or estuarine originating forms that were transported via the salt wedge up to North Baikie's mouth (e.g. cumaceans, euphausiids, *Neocalanus* sp.; Table 4) were consumed primarily by those fish that were observed in deeper water habitats (Fig. 2a). Consequently, the plankton composition in the salt wedge is more similar to the diets of hatchery coho and chinook than to the diets of wild salmonids (chum and chinook). Surface water plankton composition was more similar to the wild salmonid diets than to the diets of hatchery fish (Table 5).

Plankton abundance decreased during the course of the study. It also decreased with decreasing tidal height ($P < 0.05$; Fig. 7). Higher abundance at high tide was likely due to the influx of marine calanoids transported into the estuary within the salt wedge on the flooding tide (Table 4). The abundance of plankton was lower in the slough habitat (HNR, LNR; Fig. 7) than at the main channel stations (2–4; Fig. 1).

Discussion

The occupation of habitats by juvenile salmonids in the Campbell River estuary was influenced by species-specific reactions to many physical and biological characteristics. This finding is in accordance with the recorded reduction in interspecific competition among some salmonid species (e.g. territoriality) during smoltification and seaward migration (Keenleyside 1962; Griffith 1972; Kwain 1983; Kennedy and Strange 1982; Moyle and Vondracek 1985). Interspecific competion may therefore play a lesser role in segregating salmonid species within the estuary than within river environments, as suggested by Mason (1974), Schreck (1981), and Gibson (1983). Reduced competition has been associated with increases in habitat size and diversity (Kalleberg 1958) and with increases in food abundance (Symons 1971; Slaney and Northcote 1974), situations that occur in estuaries (Healey 1982).

Swimming ability is proportional to fish size (Brett and Glass 1973; Gray 1974), and therefore, larger fish are able to occupy deeper habitats further from shore where there is little protection from water currents (Everest and Chapman 1972; Bustard and Narver 1975; Symons and Heland 1978; Kennedy and Strange 1982) (Fig. 2 and 3). Occupying fast-flowing water may be a mechanism utilized by large fish to leave the river or estuary. However, knowing that many salmon feed within currents (Takahaski and Higashi 1984) and that faster currents present more food per unit time (Everest and Chapman 1972) (Stations 2, 3, and 4; Fig. 4 and 7), it is not

TABLE 3. Mean number of each prey type consumed per salmon collected from each region during each month. Salmon were collected by beach seine. Prey types listed are those that differed significantly in abundance among predator species or plankton samples from each microhabitat station (discriminant analysis, $P < 0.05$). Prey items such as dipterans (F), gastropods (M), calanoids, *Cyclops* sp. (F), and gravel occurred infrequently in the fishes diets (occurred at <20% of the collections) and are therefore not presented. Origin of prey is given in parentheses: F, freshwater; M, marine; E, estuary. Refer to Table 2 for an explanation of the abbreviations and sample sizes.

	North Baikie's							Painter's		
May	CMHN	COHN	COLN	WCHN	HCHN	HCLN	CULN	CMLP	COLP	HCLP
Bosmina sp. (F)	500	48	94	221	131	267	—	—	—	—
Neocalanus sp. (M)	7	31	13	30	117	26	—	8	1	6
Harpacticoida (F/E)	—	—	—	—	—	<1	—	26	22	—
Eogammarus sp. (F/E)	—	0.2	3	0.2	0.4	0.9	21	—	0.4	2
Cumella vulgaris (E/M)	—	—	—	—	—	—	—	69	29	28
Chironomidae pupae (F)	—	0.1	0.1	0.4	0.4	—	—	—	0.3	0.3
Hemiptera (F)	—	0.2	0.2	0.1	0.1	—	—	—	—	—
Zoea (M)	—	—	—	—	0.8	—	—	—	—	—
Corophium juveniles (F/E)	—	—	—	—	—	—	—	—	—	—
Euphausiidae larvae (M)	2	0.3	0.3	0.2	2	1	—	—	—	—
Gammariidae (F/E)	—	0.3	0.3	—	0.1	—	—	—	0.3	3

	North Baikie's						Painter's		
June	CMHN	CMLN	COHN	COLN	WCHN	WCLN	CMLP	COLP	WCLP
Bosmina sp. (F)	42	225	32	18	22	89	—	—	—
Neocalanus sp. (M)	76	—	3	—	17	—	0.1	—	—
Harpacticoida (F/E)	—	—	1	—	0.9	0.2	166	6	171
Eogammarus sp. (F/E)	—	—	2.3	12	0.1	—	0.1	—	0.7
Cumella vulgaris (E/M)	—	—	—	—	—	—	46	37	57
Chironomidae pupae (F)	—	—	—	—	2	—	0.1	—	4
Hemiptera (F)	—	—	—	—	4	—	—	—	—
Zoea (M)	—	—	4	—	4	0.1	0.2	—	—
Corophium juveniles (F/E)	—	—	0.2	—	0.2	0.3	—	—	—
Euphausiidae larvae (M)	15	—	1	—	5	0.9	0.1	—	—
Gammariidae (F/E)	—	—	0.2	0.1	—	—	—	—	—

	North Baikie's		Painter's
July	COLN	WCLN	WCLP
Bosmina sp. (F)	0.1	11	—
Neocalanus sp. (M)	—	—	0.6
Harpacticoida (F/E)	—	—	34
Eogammarus sp. (F/E)	11	2.1	—
Cumella vulgaris (E/M)	—	0.1	66
Chironomidae pupae (F)	0.7	1	12
Hemiptera (F)	—	0.6	0.2
Zoea	—	—	—
Corophium juveniles (F/E)	—	3	0.2
Euphausiidae larvae (M)	—	—	0.2
Gammariidae (F/E)	—	—	0.1

surprising that fish will move to areas with progressively stronger currents further from shore as they grow. For any salmonid, however, there exists a preferred upper flow rate above which energy expenditure to remain in the current outweighs the energy gain from food. While data from field studies will not allow us to obtain a direct estimate of this value, flows at North Baikie's mouth during low tide (Stations 2–4; Fig. 1) and at the surface at some stations at North

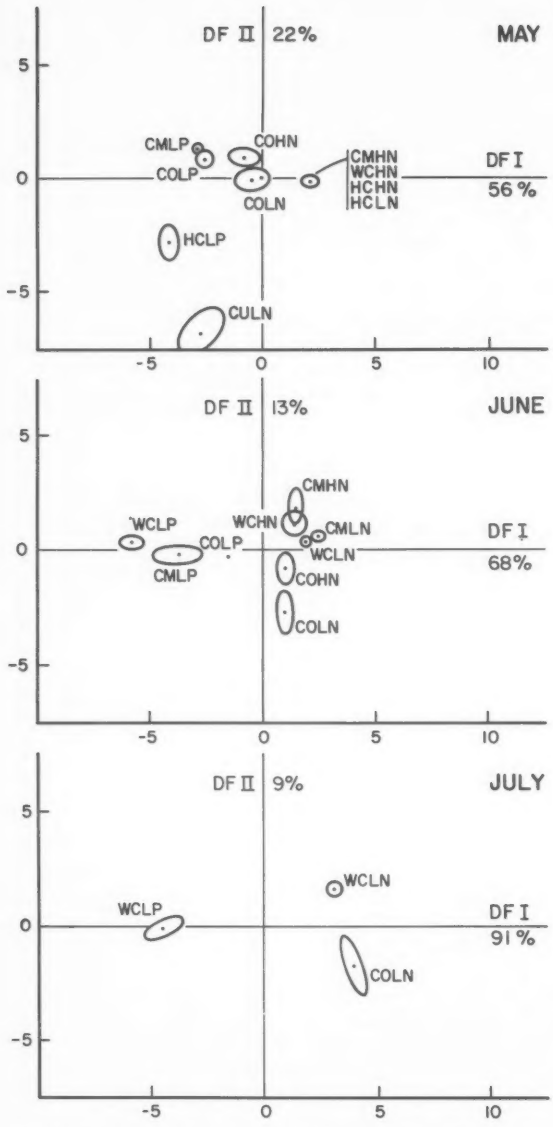

FIG. 5. Diet differences among juvenile salmon captured by beach seine from Stations 1 and 7 (Fig. 1) at high and low tide as determined using discriminant functions analysis. Data from each month are treated separately and the percentage of the total variation explained by each discriminant function is presented on the axis. Centroids of each group are surrounded by an ellipse that delimits the discriminant scores. Fish abbreviations are given in Table 2.

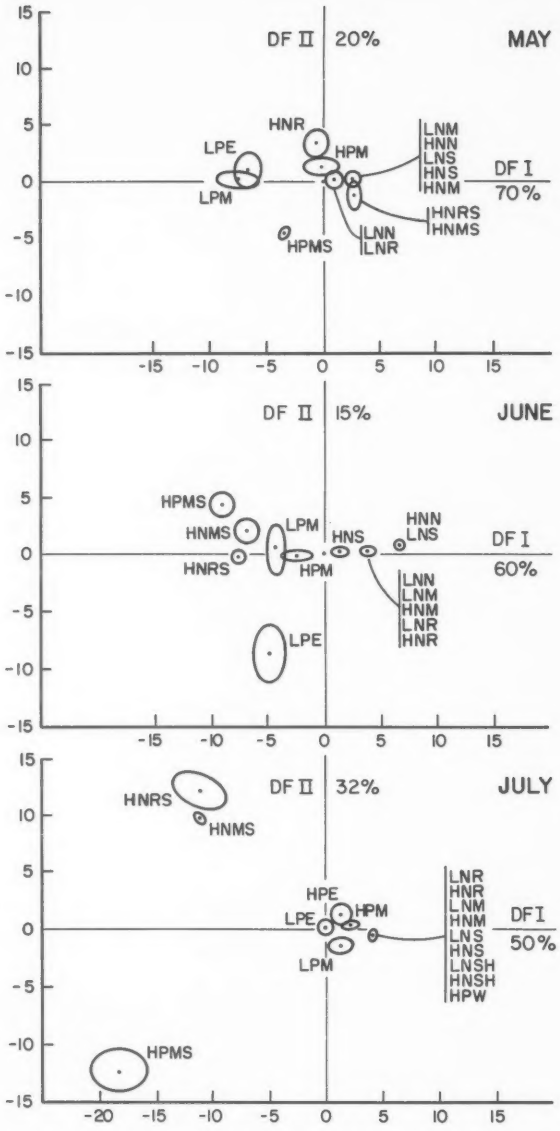

FIG. 6. Differences among microhabitat stations at both regions at high and low tide based on plankton species composition using discriminant functions analysis. Data from each month are treated separately and the percentage of the total variation explained by each discriminant function is presented on the axis. Centroids of each group are surrounded by an ellipse that delimits the discriminant scores. Station abbreviations are given in Table 2.

Baikie's mouth and Painter's Channel at high tide (particularly Station 4) appeared to be too strong to be favourable for the wild and hatchery chinook salmon (40–60 $cm \cdot s^{-1}$; Fig. 2 and 4). Flow rates of 8–35 $cm \cdot s^{-1}$, recorded frequently at North Baikie's mouth (Station 2) at high tide (Fig. 4), coincided with large concentrations of wild salmonids. Relatively large hatchery coho salmon were able to feed in areas with stronger currents at low tide, and yet maintain a speed (expressed in body lengths per second; Fig. 3) approximating that of the wild chinook. Other researchers reported swimming speeds in excess of 4.1 body lengths $\cdot s^{-1}$ (Brett 1964) or flow rates in excess of 40 $cm \cdot s^{-1}$ as being unfavourable for feeding by various species of salmonids (sockeye salmon (*Oncorhynchus nerka*), 8.5 cm, Brett and Glass 1973; brown trout (*Salmo*

TABLE 4. Mean number (m^{-3}) of plankton organisms sampled at each microhabitat during each month at low (L) and at high tide (H). Plankton organisms listed are those that differ significantly in abundance among microhabitat stations (discriminant analysis, $P < 0.05$; Fig. 6). Items such as *Mytilus* sp. (E/M) and Larvacean (M) occurred in less than 10% of the collections and are therefore not presented. Origin of the most common species in each plankton group is given in parentheses: F, freshwater; M, marine; E, estuary. Refer to Table 2 for an explanation of the abbreviations and sample sizes.

	North Baikie's mouth									Painter's		
May	L LNR	H HNR	L LNN	H HNN	L LNM	H HNM	L LNS	H HNS	H HNMS	L LPE	L LPM	H HPM
Bosmina sp. (F)	807.69	432.48	856.36	2170.62	1812.57	2097.16	1696.49	1995.45	0	7.66	6.42	650.85
Cyclops sp. (F)	99.63	287.04	54.50	97.67	73.03	88.48	89.38	139.67	58.10	6.07	1.52	156.47
Neocalanus sp. (M)	30.05	9.30	7.54	14.56	13.82	213.99	17.39	12.40	1054.33	5.87	2.45	206.54
Corophium juveniles (F/E)	0	0	1.86	0.71	1.18	0.44	1.66	0	35.44	0.80	0.60	0
Gammaridae (F/E)	0	0	4.92	0	0	1.77	0	0	2.50	7.95	0.60	0.39
Cumella vulgaris (E/M)	0	0	0	0	0	0	0	0	0	1.59	3.05	2.61
Ctenophore (M)	0	0	0	0	0	0	0	0	0	0	0	0
Euphausiidae (M)	0	0	0.33	0	0	0	0	0	2.12	0	0	0
Harpacticoida (F/E)	0	0	0	0	0	0	0	0	6.37	10.94	44.38	4.95
Chironomidae larvae (M)	0	0	0	0	0	0	0	0	0	0	0	0

	North Baikie's mouth										Painter's		
June	L LNR	H HNR	L LNN	H HNN	L LNM	H HNM	L LNS	H HNS	H HNRS	H HNMS	L LPE	L LPM	H HPM
Bosmina sp. (F)	560.87	98.20	684.52	1318.24	689.43	564.82	520.45	398.22	119.95	0	6.45	0	27.71
Cyclops sp. (F)	10.83	13.22	1.45	92.09	4.78	15.60	5.49	5.01	0	0	0	0	0
Neocalanus sp. (M)	1.87	0.23	0	0	0.20	0.27	0.59	6.19	511.64	383.64	206.45	13.69	20.19
Corophium juveniles (F/E)	0.31	0	0	0	0	0.55	5.77	0	13.82	0.14	0	0	0
Gammaridae (F/E)	0	0	0	0	0	0	0	0	0.23	0	0	0	0
Cumella vulgaris (E/M)	0	0	0	0	0	0	0	0	0	0	4.84	0.40	0
Ctenophore (M)	0	0	0	0	0	0	0	0	0.57	0	2.42	0.79	0
Euphausiidae (M)	0	0	0	0	0	0	0	0	14.62	0	4.03	0.41	0.54
Harpacticoida (F/E)	0	0	0	2.22	0.42	0.32	0	0	3.75	0	72.58	76.69	4.03
Chironomidae larvae (M)	0	0	0	0.68	1.25	0.27	0.84	0.35	0.47	0.41	0	0	0

	North Baikie's mouth										Painter's					
July	L LNR	H HNR	L LNSH	H HNSH	L LNM	H HNM	L LNS	H HNS	H HNRS	H HNMS	L LPE	H HPE	L LPM	H HPM	H HPW	H HPMS
Bosmina sp. (F)	43.19	147.69	87.43	168.40	206.94	476.29	218.53	416.56	106.11	145.04	45.43	242.08	8.98	163.23	8.87	8.77
Cyclops sp. (F)	0	0	0	0	0	4.84	0.33	7.19	0	0	15.9	0	0	1.13	0.85	0
Calanoid (M)	0	0	0	0	0	0	0	0	142.11	119.51	14.21	8.98	4.72	4.14	0.69	37.07
Corophium juveniles (F/E)	0.19	0	1.16	0	0.17	0	0.87	0	0	0	0.41	0	0	0	0	0
Gammaridae (F/E)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cumella vulgaris (E/M)	0	0	0	0	0	0	0	0	0	0	0	0	0.46	0	0	1.35
Ctenophore (M)	0	0	0	0	0	0	0	0	3.35	5.57	0	0	0	0	0	0.17
Euphausiidae (M)	0	0	0	0	0	0	0.20	0	1.71	0	0	0	0	0	0	0
Harpacticoida (F/E)	1.02	0.85	0.20	0	0	0	1.41	0	3.89	1.59	60.90	4.34	24.28	1.89	0.95	27.10
Chironomidae larvae (M)	1.14	0	1.16	0.15	0	0	4.01	0.93	0	0	0	0	0	0	0	0

TABLE 5. Matrix of values describing the similarity of diets of salmonids caught at each region at high and low tide to plankton samples collected in various microhabitats at the same time, site, and tide heights. Values are means of the similarity index calculated for data from the three collection dates. Plankton samples from Stations 2, 3, and 4 were combined to create the North Baikie's channel surface category and Stations 5, 6, and 7 to create the Painter's channel surface category.

	Tide	Chum	Coho	Wild chinook	Hatchery chinook
North Baikie's					
Channel surface	High	61	56	62	50
	Low	97	49	70	90
Slough surface	High	45	51	50	50
	Low	97	45	42	86
Salt wedge	High	24	41	32	40
Painter's					
Channel surface	High	—	—	—	—
	Low	35	23	37	7

trutta), 42 cm fork length, Shirvell and Dungey 1983; brown trout fry, Ottaway and Forrest 1983; steelhead trout, 0+ and 1+, Mundie and Traber 1983; *Oncorhynchus masou*, 10–20 cm fork length, Kamiyama 1980, cited in Takahashi and Higashi 1984).

Faced with high current velocities, there is obvious adaptive value for smaller wild salmon to seek low current refuges along the marginal areas of rivers (Lister and Genoe 1970) and estuaries. Rocks and fallen trees adjacent to the main flow are of particular value as refugia, reducing current velocity and creating back-eddies which trap potential prey (Takahashi and Higashi 1984). Fish occupying such areas can reduce energy expenditure, yet have easy access to the currents which provide food. The use of outer estuary sites at low tide to avoid strong currents upstream (Mason 1974; Iwata 1981; Healey 1982) may have less adaptive value in estuaries as large as Campbell River where tidal sloughs, marshes, and river margin cover are available as refugia from strong currents (Levy et al. 1979; Healey 1979; Levings 1982). Back-eddies created at the mouths of sloughs (shear zone, Station 8; Fig. 1) promote the mixing of deeper saline water with river water (Fig. 4). The small amounts of marine food eaten by wild salmon at high tide (Zoea, euphausiids; Table 3) may enter the upper

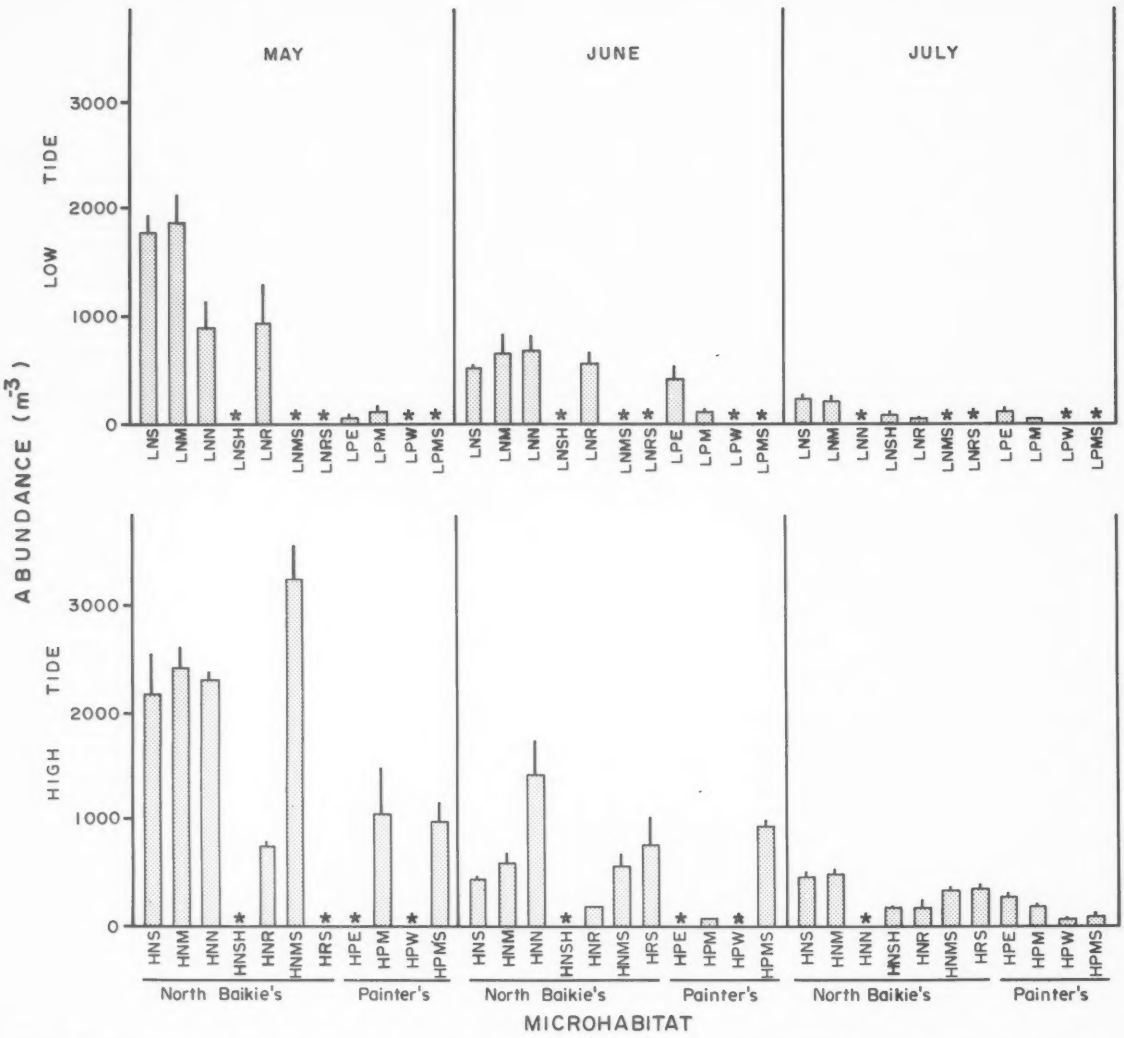

FIG. 7. Mean abundance of plankton sampled at each microhabitat station during each collection period at high and low tide. At high tide, samples from the salt wedge (>1 m water depth) were also taken and are labeled HNMS, HRS, and HPMS. Station abbreviations are given in Table 2. Vertical bars are 2 SE of the mean. Station–time combinations when no collections were made are marked with an asterisk.

water column in this manner in addition to being present in the salt wedge. Accordingly, fish may obtain food of marine origin from both surface water and deeper, more saline water below the halocline.

Inner regions of Campbell River sloughs (e.g. Station 1; LNR, HNR) may be of less value as refuge sites from high water velocity. Fewer food organisms were present there compared with other inner estuary sites (Fig. 7) and water currents may not have been sufficient to give some drift organisms (e.g. terrestrial insects) the motion that makes them attractive to juvenile salmonids (Rimmer and Power 1978). Additionally, certain Campbell River slough regions may be of less direct value as fish habitat because of low dissolved oxygen levels (e.g. log sort pond, Fig. 1, 19% saturation; Brownlee et al. 1984). Hatchery fish that frequent suboptimal sloughs throughout the year (Raymond et al. 1985; Levings et al. 1986; Fig. 2a) may be disoriented and require a period of time after release to establish correct feeding locations and methods (O'Grady 1983). Alternatively, they may lack stamina to remain in fast-flowing water and/or be attracted to the warmer water in the slough (Fig. 3 and 4). However, estuarine sloughs may be valuable habitat for wild chinook as winter refuge areas in the same manner as coho salmon and rainbow trout overwinter in freshwater sloughs (Bustard and Narver 1975; Mundie and Traber 1983).

Large salmon (e.g. hatchery coho) are more likely to be physiologically prepared for movement to the marine environment than smaller salmon (McInerney 1964). Occupation and deeper, more saline environments by larger fish (Fig. 2 and 3) may reflect their readiness to move from the estuary. Some fish

may remain only briefly and therefore spend little time feeding in salt wedge and outer estuarine habitats. This could explain why the diets of all salmon examined in this study show lower similarities with the food available in the salt wedge and at the outer estuary region than with food of the surface waters of the inner estuary (Table 5). In addition, deep-dwelling fish (coho, cutthroat, hatchery chinook) augment planktonic food resources with benthic prey (*Eogammarus* sp.; Table 3) resulting in decreased similarities between their diets and the plankton samples from the salt wedge habitat.

Juvenile salmonids within northeastern Pacific coast estuaries including the Campbell River estuary feed on a large variety of prey taxa (Healey 1982; Table 4). Interspecific differences in diets reflect differences in the potential prey items that are present in the microhabitats occupied by the fish (Gorman and Karr 1978; Williams 1981; Glova 1984; Fig. 5 and 6; Tables 3, 4, and 5). Our conclusions regarding differences in habitat utilization among the fish are supported by the results of diet analyses. As salmonids grow and move seaward, a decreased dependence on the surface layer for physiological adaptation and for food supply is reflected in a dietary shift to benthic and/or marine organisms (Table 3; Mason 1974; Greendale and Hunter 1978; Wolf et al. 1983). Daily shifts in the diets of fish captured at the inner estuary station reflect the increased availability of marine prey at high tide and freshwater prey at low tide (e.g. *Neocalanus* sp. and Euphausiidae larvae eaten by chum, chinook, and hatchery coho at North Baikie's mouth at high tide and *Bosmina* sp. eaten by wild chum and chinook at low tide; Table 3).

This study emphasizes the importance of maintaining diverse and complex estuarine habitat for the benefit of juvenile salmonids. During their estuarine residency, chum, chinook, and coho salmon utilize regions of moderate current for feeding, slough regions, stream margin cover, and backeddies for refuge and zones of increasing salinity which expose them to marine food and facilitate physiological adaptation to seawater. The importance of a specific habitat, however, cannot always be measured by the amount of time it is occupied by the fish (Larimore and Garrels 1985). High currents in regions where salmonids were rarely seen may act to dislodge benthic animals, thus making this food available to salmonids elsewhere. Infrequently used slough zones may not always have water of the quality required to support salmonids but may support the marsh vegetation on which the salmonid detrital food chain is based (Sibert et al. 1977; Healey 1979; Wolf et al. 1983). Accordingly, maintenance of a large diversity of microhabitats within the estuary may be important for the perpetuation of salmon stocks.

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Experimental Enrichment of a Coastal Stream in British Columbia: Effects of Organic and Inorganic Additions on Autotrophic Periphyton Production

Chris J. Perrin
Limnotek Research and Development Inc., 4035 West 14th Avenue, Vancouver, B.C. V6R 2X3

Max L. Bothwell
National Hydrology Research Centre, 11 Innovation Boulevard, Saskatoon, Sask. S7N 2X8

and Pat A. Slaney
British Columbia Ministry of Environment, Fisheries Branch, Research and Development Services Section, 2204 Main Mall, University of British Columbia, Vancouver, B.C. V6T 1W5

Perrin, C. J., M. L. Bothwell, and P. A. Slaney. 1987. Experimental enrichment of a coastal stream in British Columbia: effects of organic and inorganic additions on autotrophic periphyton production. Can. J. Fish. Aquat. Sci. 44: 1247–1256.

Periphyton accumulation rates and alkaline phosphatase activity were examined in reaches of the Keogh River, British Columbia, following additions of grain and inorganic fertilizer as separate treatments during spring–summer 1981. Two different levels of N and P addition were used: one to attain ambient N and P concentrations of 200 and 15 $\mu g \cdot L^{-1}$, respectively, and the other to attain 400 and 20 $\mu g \cdot L^{-1}$, respectively. Grain (rolled barley) was added monthly at 280 $g \cdot m^{-2}$. N and P additions increased chlorophyll *a* accrual rates by more than an order of magnitude. Diatoms dominated the periphyton community until midsummer. In July and most of August, the relative importance of chlorophytes increased and biomass levels declined markedly in spite of continued nutrient additions. Grain additions resulted in no detectable change in periphyton accrual, but alkaline phosphatase activity increased by 35% over control levels. These results suggest that additions of labile organic matter to nutrient-deficient coastal streams can increase autotrophic P deficiency. Based on responses of juvenile salmonids, additions of inorganic nutrients to increase autotrophic production can maximize trophic enhancement in nutrient-deficient streams.

Les auteurs ont étudié les taux d'accumulation et l'activité de la phosphatase alcaline chez le périphyton peuplant des sections de la rivière Keogh (Colombie-Britannique) après l'apport séparé de fertilisants organiques et inorganiques au printemps et à l'été de 1981. Deux teneurs différentes en N et P ont été utilisées : la première visait une concentration ambiante respective de N et de P de 200 et de 15 $\mu g \cdot L^{-1}$ et la seconde, de 400 et de 20 $\mu g \cdot L^{-1}$ respectivement. L'apport mensuel de céréales (flocons d'orge) s'est effectué à un taux de 280 $g \cdot m^{-2}$. L'apport de N et de P a entraîné une augmentation des taux d'accumulation de chlorophylle *a* de plus d'un ordre de grandeur. Les diatomés dominaient la communaute périphytonique jusqu'à la mi-été. En juillet et pendant la plus grande partie d'août, l'importance relative des chlorophycophytes a augmenté tandis que les niveaux de la biomasse ont fortement baissé malgré l'apport continu de bioéléments. L'addition de céréales n'a pas entraîné de variation discernable du taux d'accumulation du périphyton, mais l'activité de la phosphatase alcaline a augmenté de 35 % par rapport au niveau témoin. Les résultats portent à croire que l'apport de matières organiques labiles dans des cours d'eau côtiers pauvres en bioéléments peut accroître le déficit en P autotrophe. D'après les réactions des salmonidés juvéniles l'apport de bioéléments inorganiques en vue d'accroître la production autotrophe peut maximiser la stimulation des niveaux trophiques dans les cours d'eau pauvres en bioéléments.

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Techniques for enhancing wild stocks of anadromous salmonids on Canada's Pacific coast include efforts to increase food supply for juveniles that rear in freshwater. Potential enhancement is based on evidence presented by Ricker (1962) and later by Peterman (1982) and Bilton et al. (1982) showing that on average, larger smolts have greater marine survival and produce less variability in adult returns than smaller smolts. Hence, increased food supply must lead to enhanced growth of juveniles and increased size of smolts to be potentially useful.

Two general approaches to increase food supply to juvenile salmonids have been considered in British Columbia. One is exemplified by the Lake Enrichment Program (LEP) (Hyatt and Stockner 1985; LeBrasseur et al. 1978), a group of projects that include both experimental and operational fertilizer additions to coastal lakes for the enhancement of sockeye salmon (*Oncorhynchus nerka*). In the LEP, inorganic N and P are added to stimulate trophic productivity. The other approach considered for enhancement of stream-rearing salmonids has been enrichment using particulate organic matter (POM)

(Mundie et al. 1983). Unlike the LEP, stream enrichment using POM has not been developed to include streams other than that used for initial experiments.

A study was conducted in the upper Keogh River, British Columbia, in 1981 to compare the effects of organic and inorganic enrichment on food supply and growth of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). This paper compares changes in P deficiency and productivity of benthic algae in adjacent stream reaches enriched with POM and inorganic fertilizer in spring and summer.

Study Site

The Keogh River flows for 32 km from a 129-km^2 watershed located at 127.4°W and 50.6°N. Headwaters originate in the Vancouver Island mountain range and the river discharges into Queen Charlotte Strait on the northeast side of Vancouver Island (Fig. 1). Discharge is highly variable since recording began in 1975; the minimum flow has been 0.1 $m^3 \cdot s^{-1}$ (1978) and the maximum was 225 $m^3 \cdot s^{-1}$ (1975). Mean summer discharge is 1.6 $m^3 \cdot s^{-1}$ (1976–80). Flow patterns are "flashy" and correspond directly to precipitation. Mean annual total precipitation is 173 cm (Environment Canada Weather Office, Port Hardy), with most occurring as rain in autumn and winter. Snowpack occurs only at higher elevations of the Keogh watershed. During midsummer there is a dry period of about 4 wk when the river has a stable low flow.

Our study was conducted in Reach Z (Fig. 1), a 5-km section at the upstream end of the Keogh River. Reach Z has an average width in summer of about 6 m, a gradient of 0.84%, and a mixed gravel and cobble bottom. Habitat proportions by area are 40% riffle, 26% pool, and 34% run (Ward and Slaney 1979). Chemical records (Ward and Slaney 1979) show that average annual concentrations of both anions and cations are low, giving a filterable residue of <40 mg$\cdot L^{-1}$ and a specific conductance of 2.0–3.0 mS$\cdot m^{-1}$. Total alkalinity and pH during spring and summer average 7.0 mg $CaCO_3 \cdot L^{-1}$ and 6.9, respectively. Soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) concentrations are about 1 and 5 $\mu g \cdot L^{-1}$, respectively (Perrin and Johnston 1985). Ammonia is rarely detectable and $NO_3^- + NO_2^-$-N rarely exceeds 15 $\mu g \cdot L^{-1}$. Riparian growth is dominated by red alder (*Alnus rubra*), salal (*Gaultheria shallon*), willow (*Salix* spp.), and several sedges and grasses. Tree species include Western hemlock (*Tsuga heterophylla*), red cedar (*Thuya plicata*), and sitka spruce (*Picea sitchensis*). These species occurred mainly as early seral regeneration (up to about age 20) because of forest harvesting over the past 30 yr. The forest canopy was not closed in Reach Z.

Materials and Methods

Stream flow, light, and temperature were continuously measured at the W103 bridge location (Fig. 1). Water level was measured using a Leupold and Stevens (Beaverton, Oregon) model F water level recorder. A calibration curve for calculating stream flow was determined by rating periodic flow measurements to gauge height. Flow was measured by multiplying wetted cross-sectional area by average current velocity measured with a Marsh McBirney (Rockville, Maryland) electromagnetic velocity sensor. Light intensity was monitored using a LI-COR (Lincoln, Nebraska) printing integrator L1-550B equipped with a quantum sensor. The sensor was located at a site 1 km downstream of the W103 bridge that was typical of the experimental reach in that an overhead forest canopy was absent, but there was lateral shading in early morning and late afternoon. A Ryan (Kirkland, Washington) model J-90 submersible thermograph was used for continuous recordings of stream temperature, and the instrument was calibrated weekly.

Within 2 km of Reach Z, four 300-m experimental sections were designated, each having similar aspect, cover, gradient, and substrata characteristics (Fig. 1). The upstream-to-downstream layout was as follows:

(1) Control.

(2) Treatment 1 (T1): addition of 280 g rolled barley$\cdot m^{-2}$ applied once per month for the period May 16 through September 15, 1981.

(3) Treatment 2 (T2): inorganic fertilizer (34-0-0 plus 11-55-0) addition to achieve ambient concentrations of 15 μg $P \cdot L^{-1}$ and 200 μg $N \cdot L^{-1}$ from May 14 through September 15, 1981.

(4) Treatment 3 (T3): inorganic fertilizer (34-0-0 plus 11-55-0) addition to achieve ambient concentrations of 20 μg $P \cdot L^{-1}$ and 400 μg $N \cdot L^{-1}$ from May 14 through September 15, 1981.

Adjacent treatments were separated by a 300-m buffer zone where no treatment was applied. Two fertilizer treatments were used in the present study to determine if periphyton accumulation rates continued to increase at higher levels of enrichment.

Fertilizer was dispensed using two equally spaced DF1-A Sweeney feeders (Boerne, Texas) in each of the fertilized sections (Fig. 1). Feeders were equipped with electronic timers set to dispense approximately 700 g of fertilizer once per hour. Input rates were adjusted to varying flow conditions.

In T1, rolled barley (total C = 39.24%, H = 6.59%, total N = 1.88%, total P = 0.40% based on air dry weights) was applied evenly by hand over the stream substratum after soaking the grain for about 30 min in plastic containers. Soaking prevented the grain from floating downstream after application. Flow conditions distributed the grain evenly in runs but deposited clumps in pools. In riffles, grains lodged into interstitial spaces within gravel and cobble.

Within each of the control and treatment sections, two 15-m-long riffles were selected for sampling of dissolved nutrients, periphyton accumulation rates, species composition, and alkaline phosphatase activity. In fertilized sections, these riffle sites were 100 m downstream of each fertilizer dispenser. These equal distances were intended to result in similar dissolved inorganic nutrient concentrations at each sampling riffle. By defining the spatial confines of our sampling units as specific riffle sites that received similar levels of enrichment of each treatment, riffle sites within treatments can be considered replicates.

Sampling at each riffle site was initiated April 10, 1981. Water samples to be used for dissolved nutrient measurements were collected weekly and passed through 0.45-μm Millipore filters. All samples were packed in coolers and shipped to Vancouver for analysis within 48 h of sample collection. $NO_3^- + NO_2^-$-N was determined using the cadmium reduction method of Strickland and Parsons (1972). Ammonia was measured colorimetrically, as described by Traversy (1971), and SRP was determined by the ascorbic acid reduction method of Murphy and Riley (1962).

Periphyton was sampled from four replicate 30 × 30 ×

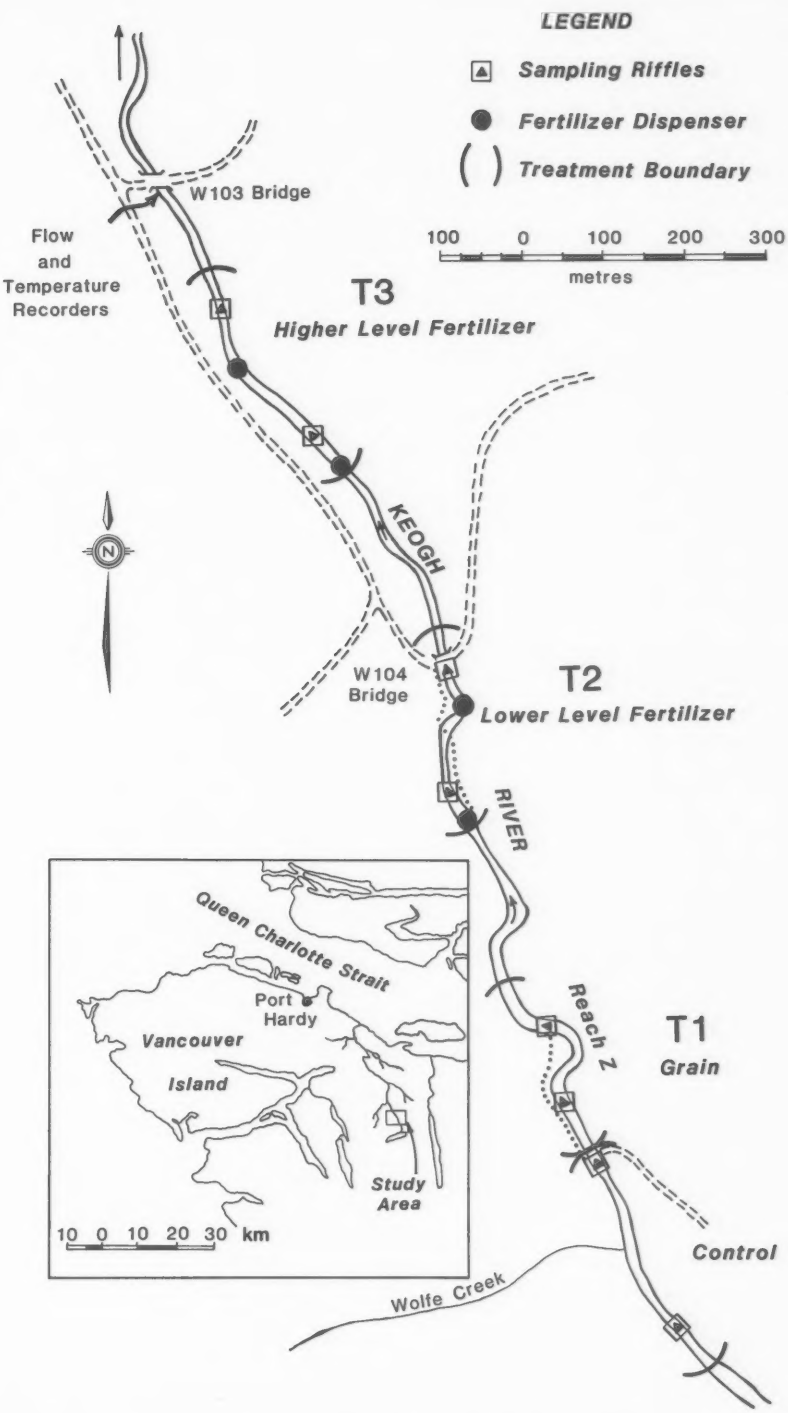

FIG. 1. Site detail of study reaches in Keogh River Reach Z.

TABLE 1. Photosynthetically active radiation (PAR) during each periphyton accumulation series.

Periphyton accumulation series	Dates	PAR (Einst·m^{-2}·d^{-d}) Mean	Max	Min
1	Apr. 10–May 15	—	—	—
2	May 16–May 28	—	—	—
3	June 24–July 9	57.0	70.3	37.14
4	July 12–Aug. 13	49.29	69.06	26.84
5	Aug. 15–Sept. 22	46.82	59.13	38.50

0.64 cm sheets of styrofoam-DB (Snowfoam Products, El Monte, California) substrata located in each riffle. The styrofoam was attached to a 0.7-cm-thick acrylic surface which was bolted to a 5.1 × 30.5 × 30.5 cm concrete block embedded in the stream bottom. All plate assemblies were located where the current velocity was approximately 40 cm·s^{-1} and water depth above the styrofoam surface was 10 cm. When flows and water depth changed, sampling substrata were moved to maintain the intended physical conditions.

The styrofoam was sampled for chlorophyll *a* every 4–7 d. On each date, one sample was collected from each replicate plate by removing a core with the open end of a 12-dram (8.55-cm^2 core area) plastic vial. Acidification was prevented by adding a few drops of $MgCO_3$. Samples were stored in the dark at −20°C for up to 1 wk before extraction of the cores in 90% acetone and analysis using the spectrophotometric technique of Strickland and Parsons (1972).

Five time-course sequences of chlorophyll *a* accumulation in each section were made from April through September. The first series was run before treatments began, and the four others were run during treatment (dates shown in Table 1). As an aid in detecting differences between treatments, an analysis of covariance was used to test for differences between slopes of the linear part of the increase in chlorophyll *a* concentrations. All slopes were determined by least squares analysis. It should be noted that although these slopes represent the net rate of periphyton accumulation, they are not the same as specific growth rate measurements. Care was taken to install the artificial substrata at similar depths and current velocities, but site-to-site variation in passive settlement and insect grazing were uncontrolled.

At the end of each accumulation period, two additional cores were collected from each plate: one for a determination of alkaline phosphatase activity (APA) and the other for taxonomic examination. Since APA is inversely related to the supply of external P (Perry 1972), APA was considered useful for evaluating P deficiency. Cores for APA analysis were frozen at −20°C in the dark until analysis using 100 μm of 3-*O*-methylfluorescein phosphate (MFP) as the reactive substrate (Healey and Hendzel 1979). These values were normalized to chlorophyll *a* determined from subsamples of the same homogenized core material used for the APA analysis. Taxonomy samples were preserved in Lugol's acetate solution and stored at room temperature for up to 3 mo before examination. The relative abundance of each algal class or phyla and genera was determined from periphytic material which was scraped from the styrofoam with a stiff-bristle toothbrush. Samples were allowed to settle in Utermohl chambers for at least 8 h. Several transects were then examined at 500× magnification to determine the percent composition, as described by Northcote et al. (1975). Extracellular products (e.g. gelatinous stalks) were included in the estimate. Reference works used for identification were Hustedt (1930), Cleve-Euler (1951–55), Prescott (1962), and Patrick and Reimer (1975).

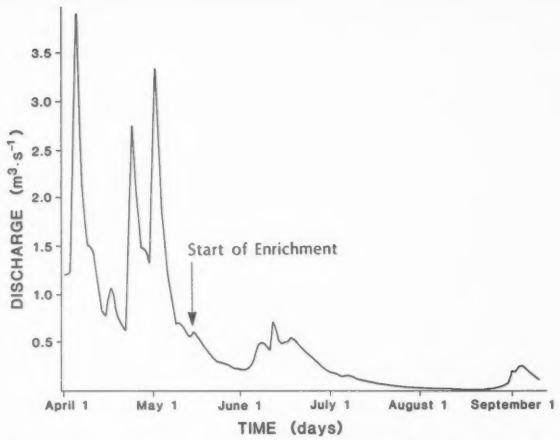

FIG. 2. Keogh River flow rates at the W103 location of Reach Z during spring and summer 1981. Stream enrichment began on May 16.

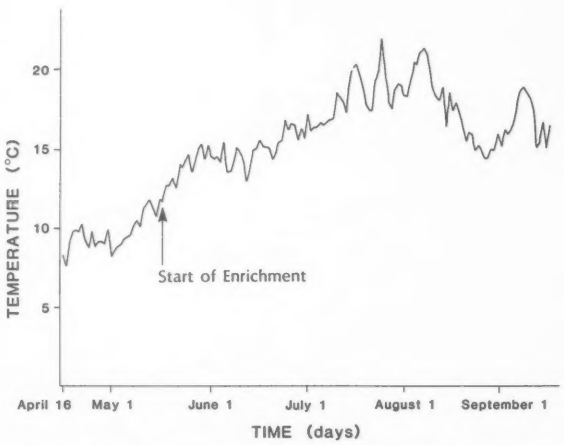

FIG. 3. Keogh River water temperature at the W103 location of Reach Z during spring and summer 1981.

Results

Physico-chemistry

Physical data collected in Reach Z were typical of dry summer conditions without freshets. Average stream flow from April to September was 0.54 m^3·s^{-1}, and a low summer flow of approximately 0.2 m^3·s^{-1} was reached by late July (Fig. 2). Photosynthetically active radiation (PAR) (Table 1) ranged from an average high value of 57 Einst·m^{-2}·d^{-1} when recording began in late June to a lower level of approximately 47 Einst·m^{-2}·d^{-1} by the end of July. Water temperatures (Fig. 3) were <10°C in late April, peaked at 22°C during early

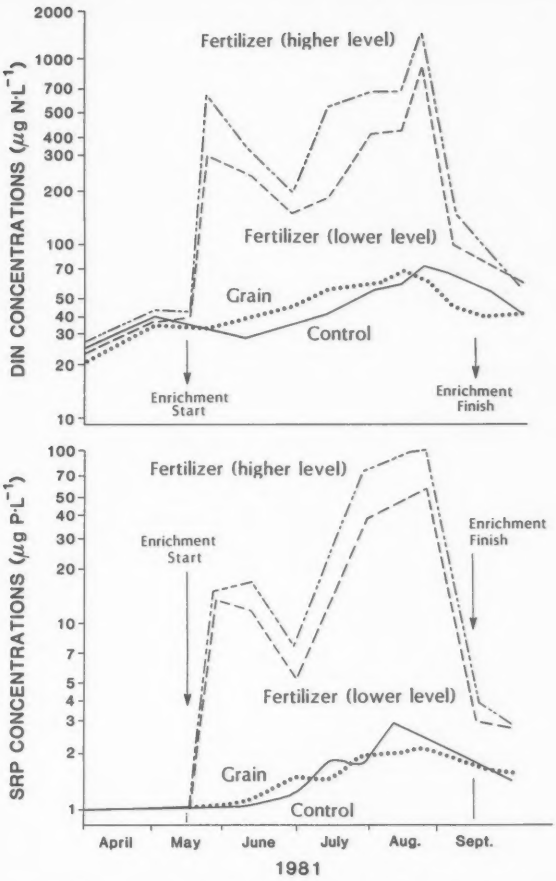

FIG. 4. Macronutrient concentrations before and during enrichment in Keogh River Reach Z for each treatment and control section. Ordinate scales are logarithmic. Nitrogen concentrations are as NO_3^- + NO_2^- + NH_3 + NH_4^+-N (DIN).

August, and were 17°C at the termination of the study in September.

Initial background dissolved nutrient levels in the river were very low (Fig. 4): SRP was ≤ 1 $\mu g \cdot L^{-1}$, the average NO_3^- + NO_2^--N level was 10 $\mu g \cdot L^{-1}$ and NH_3 + NH_4^+-N was undetectable (<5 $\mu g \cdot L^{-1}$). These low concentrations were unchanged by the grain treatment. However, some small changes in background nutrient levels were noted during the study (Fig. 4). SRP, NO_3^- + NO_2^--N and NH_3 + NH_4^+-N increased to 3, 60, and 10 $\mu g \cdot L^{-1}$, respectively, by August. Thereafter, concentrations declined to reach pretreatment levels by mid-September. In fertilized sections, nutrient concentrations rarely declined below the intended treatment level, but there was considerable temporal fluctuation due both to periodic feeder malfunction and changes in stream flow (Fig. 4). Average SRP and dissolved inorganic nitrogen (DIN) concentrations were 15 and 250 $\mu g \cdot L^{-1}$, respectively, in T2 and 25 and 480 $\mu g \cdot L^{-1}$, respectively, in T3.

Although there was no movement of grain into sections downstream of T1, analysis of water samples collected upstream of T3 immediately after fertilization started showed that elevated N and P levels from T2 continued into T3. Consequently, fertilizer applications in T3 were constantly adjusted to prevent nutrient concentrations from exceeding the intended level. Similar adjustments were made to feeders within treatments to ensure that replicate riffles received a similar level of enrichment. Macronutrient concentrations up to threefold greater than those measured in the control were also detected in periodic samples collected 2 km downstream of the study site.

Periphyton Accrual

Accumulation rates of periphyton prior to treatment (Fig. 5a) were similar in all sections (0.10 mg Chl $a \cdot m^{-2} \cdot d^{-1}$, $p < 0.05$). Chlorophyll *a* standing crops never exceeded 10 $mg \cdot m^{-2}$, and the mean was only 4.72 $mg \cdot m^{-2}$ (SE = 0.31). This level is typical of periphyton abundance which has been reported for other nutrient deficient streams at 10°C (Stockner and Shortreed 1978; Peterson et al. 1983). In late May, accumulation rates in the control and T1 more than tripled to approximately 0.35 mg Chl $a \cdot m^{-2} \cdot d^{-1}$).

Following the start of enrichment, stimulatory effects of inorganic fertilizer additions were clearly visible. Chlorophyll *a* accumulation rate in T2 increased by more than an order of magnitude to 4.1 $mg \cdot m^{-2} \cdot d^{-1}$, and that in T3 (8.6 $mg \cdot m^{-2} \cdot d^{-1}$) was more than double T2. Within 15 d the average chlorophyll *a* standing crop increased from <10 to approximately 90 $mg \cdot m^{-2}$ in T2 and 150 $mg \cdot m^{-2}$ in T3. However, no differences in periphyton biomass were detectable between grain (T1) and control sections ($p < 0.05$).

In series 3 (Fig. 5b), the spread of rates and standing crop between T2 and T3 narrowed. Chlorophyll *a* accumulation rate in T3 increased by approximately 20% to 11.0 $mg \cdot m^{-2} \cdot d^{-1}$, but T2 rates doubled to 8.8 $mg \cdot m^{-2} \cdot d^{-1}$ from the earlier measurements, resulting in a marked increase in maximum chlorophyll *a* levels in T2 after 15 d compared with the same duration in series 2.

In series 4, temperature reached a maximum, flow reached a minimum, and treatment effects changed markedly (Fig. 5c). Chlorophyll *a* accumulation rates in the control and T1 were again similar to previous levels, but those in both T2 and T3 declined by up to 90%. In addition, previous differences between T2 and T3 were no longer detectable ($p < 0.05$). Although significant differences in both accumulation and chlorophyll *a* standing crop remained between fertilized sections and control, the order of magnitude difference seen in series 3 had declined to only a threefold difference. By mid-July, sloughing of large mats of accumulated biomass was visibly obvious despite continuous additions of N and P. In series 5, there was a renewed response to elevated nutrients that corresponded with declining temperature and light levels. Although the large responses observed in series 3 did not reoccur, accumulation rates of chlorophyll *a* in T2 (2.8 $mg \cdot m^{-2} \cdot d^{-1}$) and T3 (5.4 $mg \cdot m^{-2} \cdot d^{-1}$) increased up to an order of magnitude greater than control levels (series 5, Fig. 5d). However, over the latter 15-d period in series 5, maximum biomass in T3 was only 60% of that during a similar length of time in series 3, and biomass in T2 only reached approximately 50 mg Chl $a \cdot m^{-2}$, about half of the response in series 3.

Periphyton Taxonomy

Diatoms comprised most of the biomass of the attached algal

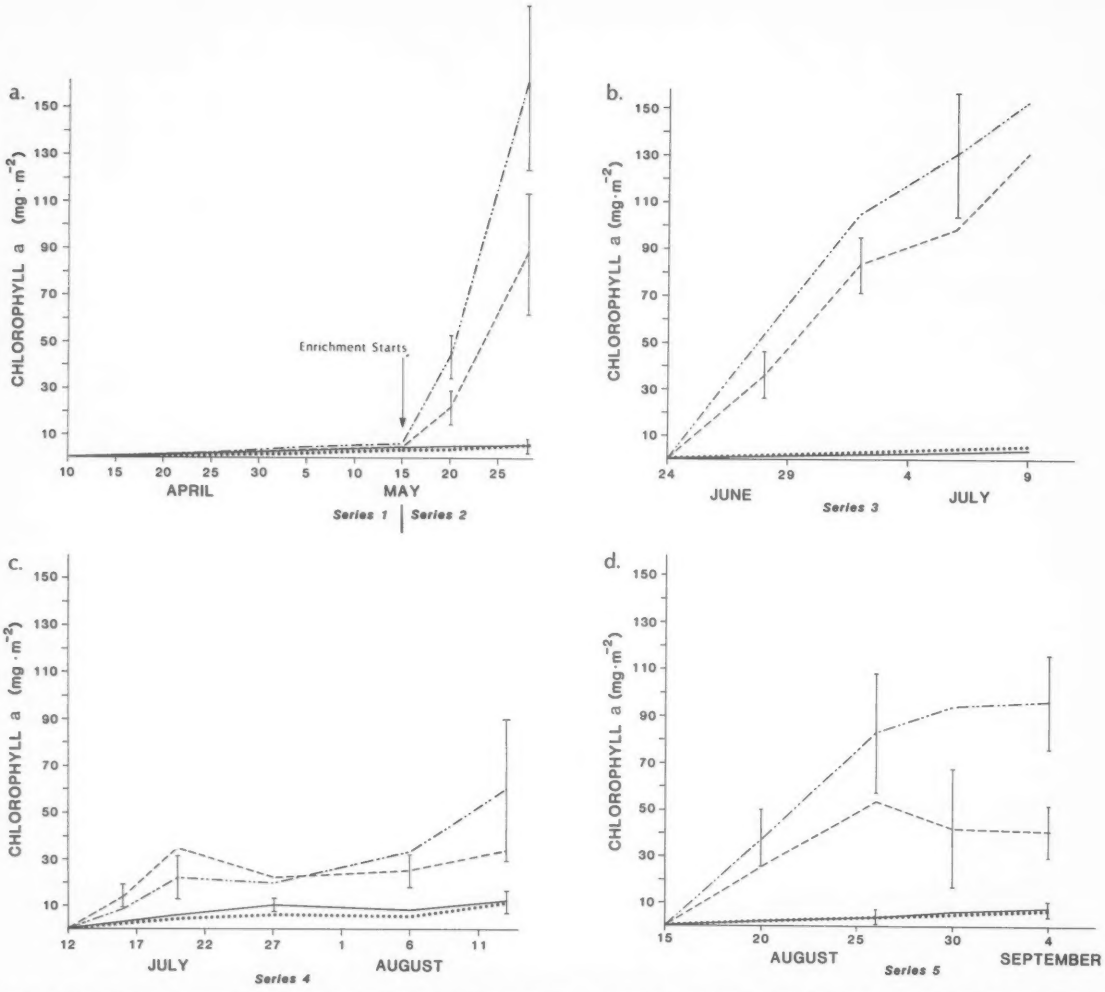

FIG. 5. Chlorophyll *a* accumulation on in situ styrofoam substrata in control (—), grain (···), lower level fertilizer (---), and higher level fertilizer (—·—·—) treatments during each of five accumulation series. Note that abscissa scales are different between series. (a) pretreatment (series 1) and during treatment (series 2); (b) series 3; (c) series 4; (d) series 5. Error bars represent 95% confidence intervals.

community in all sections up to mid-July (Fig. 6). *Tabellaria* and *Synedra* were most common. *Achnanthes* spp. numerically comprised up to 50% of some samples. Other genera including *Fragilaria* and *Diatoma* were of relatively minor importance. Planktonic forms of chlorophytes were also present. Bluegreens were a very small component of the attached community.

By early July (series 3), stream flows were steadily declining and there was an increase in the diversity of genera that are typically found in slow-water environments. In fertilized sections the filamentous chlorophyte *Ulothrix* was abundant, turning those areas a vivid green colour. In series 4, the relative abundance of diatoms declined markedly and the proportion of both tychoplanktonic and euplanktonic chlorophytes increased in all sections (Fig. 6). *Ulothrix* virtually disappeared and the diversity and proportionate importance of desmids and other pool-dwelling genera was particularly striking. *Spirogyra* replaced *Ulothrix* in the fertilized reaches. At this time, the proportionally smaller diatom community was also made up of different genera including *Cymbella* and *Cyclotella*. The bluegreen *Oscillatoria* remained insignificant in control and grain treatments but did increase by 2–5% in fertilized areas. During series 5 the diatom flora regained its relative dominance in T3 (Fig. 6), but even as late as September 22, a wide diversity of desmids and other planktonic genera remained common.

APA

Before addition of nutrients on May 16, APA values in all sections ranged from approximately 70–145 nmol MFP·μg Chl $a^{-1}\cdot h^{-1}$ (Fig. 7). Eleven days following the commencement of treatments, APA in both T2 and T3 declined to levels indicative of no P deficiency (Healey and Hendzel 1979). Simultaneously, APA in the control and grain-enriched sections increased significantly to levels suggesting extreme P

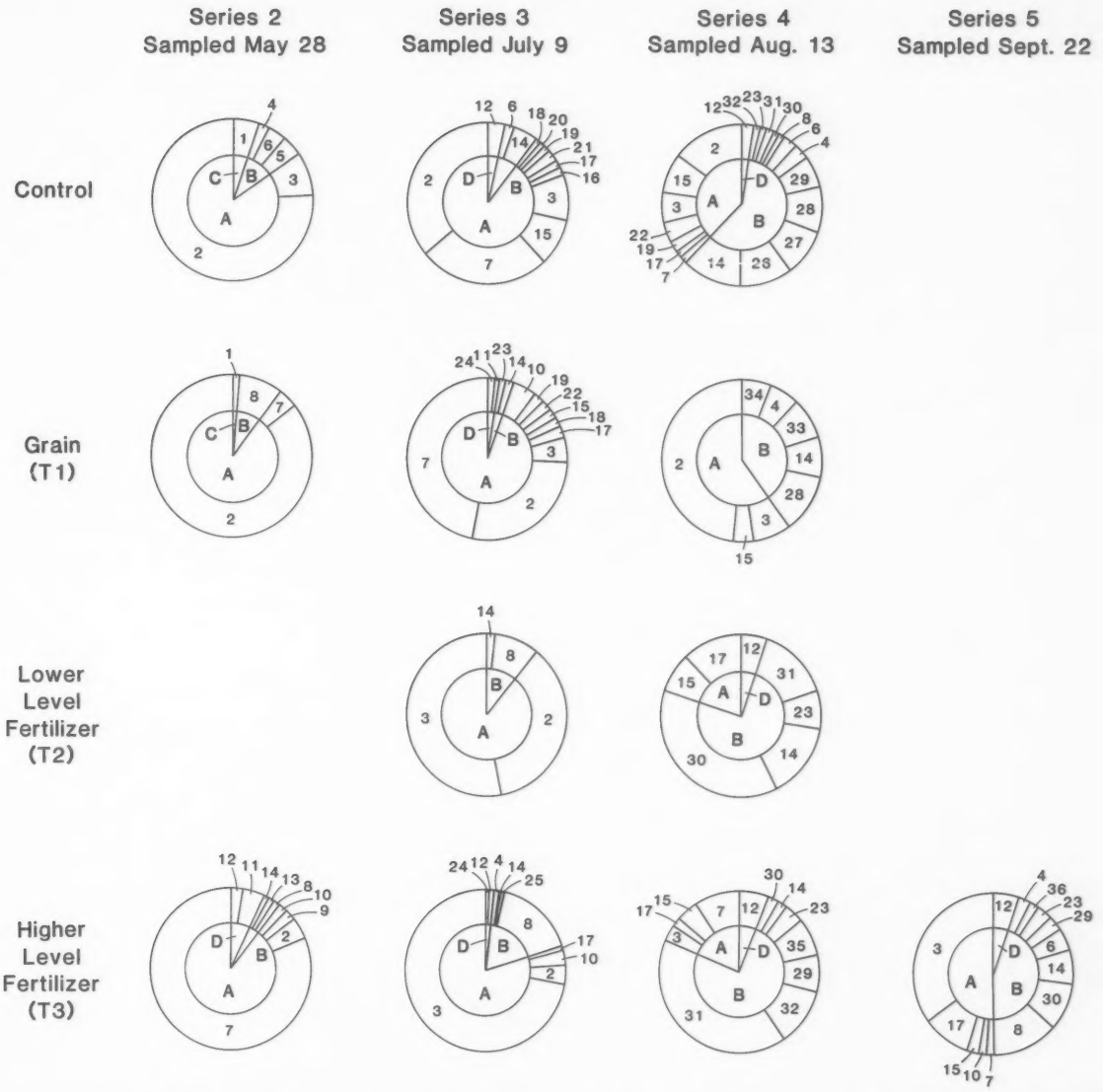

FIG. 6. Proportions of algal classes or phyla and genera on styrofoam substrata at the end of accumulation series 2–5. Data are missing for the control, T1, and T2 in series 5 and for T2 in series 2. The inner circle shows percent composition of Bacillariophyceae (A), Chlorophyta (B), Chrysophyta (C), and Cyanophyta (D). Numbers in the outer circle correspond to the genera listed below. Divisions in the outer circle represent percent composition of these genera within the class or phyla. 1, *Dinobryon*; 2, *Tabellaria*; 3, *Synedra*; 4, *Staurastrum*; 5, *Penium*; 6, *Closterium*; 7, *Achnanthes*; 8, *Ulothrix*, 9, *Diatoma*; 10, *Fragillaria*; 11, *Chamoesiphon*; 12, *Oscillatoria*; 13, *Zygnema*; 14, *Cosmarium*; 15, *Navicula*; 16, *Melosira*; 17, *Gomphonema*; 18, *Nitzschia*; 19, *Eunotia*; 20, *Denticula*; 21, *Cyclotella*; 22, *Cymbella*; 23, *Ankistrodesmus*; 24, *Anabaena*; 25, *Trachlmonas*; 26, *Teilingia*; 27, *Tetraedron*; 28, *Bulbochaete*; 29, *Chlamydomonas*; 30, *Scenedesmus*; 31, *Spirogyra*; 32, *Selenastrum*; 33, *Spondylosium*, 34, *Pediastrum*; 35, *Sirogonium*; 36, *Mougeotia*.

deficiency ($p < 0.05$). For the duration of summer, APA values in the nutrient-enriched sections remained very low. Values in T3 were no different from those in T2 ($p < 0.05$). High APA values in the control and grain treatment areas persisted throughout the summer. APA in the grain enrichment was always significantly higher than in the control by a 35% margin ($p < 0.05$).

Discussion

Periphyton Accrual

Accumulation rates of periphytic algae in the Keogh River clearly increased with additions of N and P. In T2 and T3, increases were at least in part due to elimination of P deficiency, a finding supported by other studies on nutrient defi-

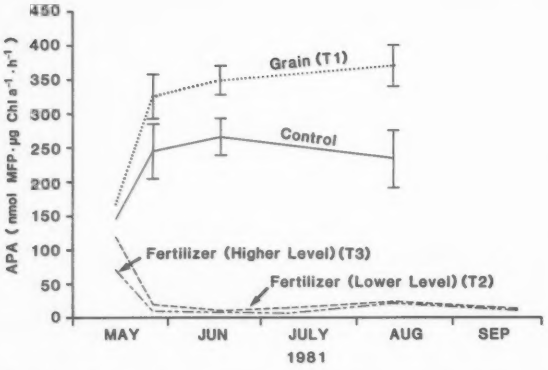

FIG. 7. Alkaline phosphatase acitivty (APA) in control (—), grain (···), lower level fertilizer (---), and higher level fertilizer (-·-·-) treatments section of Keogh River Reach Z during summer 1981.

ciency and growth of benthic algae. Bothwell (1985), for example, found that P concentration limits algae production in the Thompson River system, British Columbia, and that SRP concentrations of only 3–4 $\mu g \cdot L^{-1}$ are sufficient to saturate P-limited growth rates. Recent studies by M. L. Bothwell (National Hydrology Research Centre, Saskatoon, Sask., unpubl. data) suggest that growth may be saturated at even lower concentrations of P. In a study at Carnation Creek, Vancouver Island, where streamside troughs were used to examine periphyton accumulation, Stockner and Shortreed (1978) found that by increasing SRP levels from <1 to 8–10 $\mu g \cdot L^{-1}$, chlorophyll *a* levels increased by more than 10 times. By adding inorganic N, as well as P, a further doubling of biomass to >200 mg Chl $a \cdot m^{-2}$ was reported. In laboratory streams, Horner et al. (1983) found that SRP levels of 15–25 $\mu g \cdot L^{-1}$ produced what were considered "nuisance" conditions of algal biomass at 100–150 mg Chl $a \cdot m^{-2}$. Periphyton biomass in excess of 100 mg Chl $a \cdot m^{-2}$ were common in T2 and T3, particularly in series 3, but the increase from control levels was not perceived as a negative impact, since mean weights of juvenile salmonids increased by up to 80% due to treatment (P. A. Slaney, B.C. Ministry of Environment and Parks, Fisheries Branch, University of British Columbia, Vancouver, B.C., unpubl. data).

No change in periphyton accumulation was detectable in response to the grain addition. However, APA was greater in T1 than in the control by 35% ($p < 0.05$). Hence, although differences in periphyton accumulation between T1 and the control were not detectable using the artificial substrata, the addition of POM may have reduced specific growth rates due to higher P deficiency. This difference in P deficiency between the control and T1 will be further discussed below.

In three of the four incubation series when fertilizer was added, algal accumulation rates were significantly greater in T3 than in T2. Higher accumulation rates at higher concentrations of N and P are consistent with findings of Horner and Welch (1981) showing that biomass of stream periphyton may remain P limited at concentrations exceeding 40 $\mu g \cdot L^{-1}$. Evidence that P-limited growth of periphytic diatoms saturates at only 3–4 $\mu g \cdot L^{-1}$ (Bothwell 1985) suggests that diffusion limitation of P may be important in dense algal mats. Thus, relatively high P concentrations are required to saturate rates of biomass accrual. The possibility of differences in P deficiency in algal mats between T2 and T3 is, however, at odds with APA data that suggest that periphyton in both T2 and T3 were P replete.

One explanation for this disparity may be the confounding effect of passive settlement on measurements of algal accumulation rates, an effect that was originally described by Bothwell and Jasper (1983). Algal settlement rates (milligrams chlorophyll *a* per square metre per day) are, in part, controlled by the concentration of suspended algae (milligrams chlorophyll *a* per cubic metre) that has sloughed from upstream substrata. Hence, the large increase in attached biomass in T2 and presumably that in the buffer reach between T2 and T3 may have increased algal drift and subsequent settlement downstream in T3. Settlement in T3 would have resulted in measurements of greater biomass accumulation rates in T3 even if biomass accrual due to growth within T3 was similar to that in T2. Another explanation for differences in periphyton accrual between fertilized reaches relates to differences in rates of insect grazing. Although Perrin and Johnston (1985) reported no significant difference in the biomass of collector or scraper invertebrates between T2 and T3, invertebrate production may have differed and impacted on accrual of periphyton biomass.

A most striking feature of the periphyton accrual data was the decline in accumulation rate in T2 and T3 beginning in early to mid-July despite continuous enrichment. There are a variety of explanations to be considered related to biological and physical factors.

First, July was a period of highest irradiance and some of the highest temperatures were also recorded at this time. Because of very low flows, water depths in many areas, particularly riffles, where <10 cm. These are conditions when ultraviolet toxicity may be suspected to limit growth (Soeder and Stengel 1974) due to photodestruction of chlorophyll (Meeks 1974) and inactivation of chloroplasts (Cock 1982). Recently, M. L. Bothwell (unpubl. data) has clearly shown that periphyton growth rates can be inhibited under natural UV exposure during summer months at the Thompson River.

A second possibility is change in insect grazing activity. Lamberti and Resh (1983) have shown that patterns of periphyton accrual follow a fluctuating pattern of biomass increase and decrease related to the competitive spacing of insects. Hart (1981) also suggested that insect larvae exploit periphyton resources, and Jacoby (1985) found that periphyton biomass was about four times less in enclosures containing high densities of snails compared with periphyton biomass in exclosures where snail densities were 10 times lower. Significant changes in cropping rates seem unlikely in our data, however, because of differences in timing between the decline in periphyton accumulation rates and temporal responses by collector and grazer insects that dominated the benthic community. Perrin and Johnston (1985) have shown that invertebrate biomass did not increase ($p < 0.05$) until late August, a time when algal accumulation rates were returning to higher levels. Results, however, may have been confounded with cropping of invertebrates by fish which were growing rapidly during the decline in algal biomass.

A third explanation is algal cell lysis caused by bacteria, fungi, and/or actinomycetes. There is a broad correlation between bacteria activity and algal production (Hobbie and Rublee 1977) in a variety of aquatic ecosystems. Hence, one would expect bacterial activity to have increased in response to a greater standing crop of algae in T2 and T3. The same would

also occur due to loading of organic matter in T1. Although the presence of any lytic bacteria was not examined in our study, Daft et al. (1975) has shown that lysis of a cyanophyte can be extremely rapid after an initial chemotaxic response followed by bacteria attachment to the cell wall. Also, *Scenedesmus* spp. can become infected with a bacterium that digests cells from the inside out (Schnepf et al. 1974). It appears that all lysis can be selective to species or stages of cell division that have weak cell wall structures (Gunnison and Alexander 1975).

A fourth explanation relates to seasonal shifts in micronutrient supply. Silica concentrations were determined on an irregular basis but ranged between 4000 and 4500 $\mu g \cdot L^{-1}$, well above a level considered to limit growth of a typical planktonic diatom (Kilham 1975). Although most micronutrients were not measured, any decline in concentrations could have limited growth rates and, in particular, contributed to the shift in community taxonomy shown in Fig. 6 (cf. Patrick 1978).

Finally, the effects of flow cannot be ignored. Diatoms examined from all sections are considered typical of the fast-flowing habitats that existed prior to mid-July (Smith 1950; Prescott 1962). The increase in importance of planktonic genera occurred at the same time of minimum flow when quiescent backwater and pools were common. This shift in the physical environment may have selectively inhibited diatom growth. However, the specific process involved is difficult to resolve. Nutrient supply at the cell boundary can decline at low flow and reduce rates of nutrient uptake and respiration (Whitford and Schumacher 1964), but Lock and John (1979) suggested that this boundary effect may not be important in nutrient-replete conditions as were characteristic of T2 and T3.

APA

Regardless of these temporal trends in biomass accumulation, differences in the physiological status of periphyton remained constant between sections. Very low APA levels in T2 and T3 suggest continuous P sufficiency. Of even greater interest, however, is the relative increase in APA due to additions of grain in T1. There are three possible explanations for this increase in APA over control levels. The most obvious is that algae may have become more P limited because of increased competition from bacteria for a limited supply of ambient P. Compared with most algae, heterotrophic bacteria are known to be superior competitors for phosphate (Rhee 1972; Brown et al. 1981). When heterotrophs are not limited by organic carbon, they can outcompete algae under P-limited conditions (Brown et al. 1981; Schindler 1975). Competition for P increases P limitation in the autotrophs and is consistent with our observation of an increase in APA following POM additions. A second explanation is that if heterotrophic demand for P did increase as a result of POM additions, then any increase in bacteria biomass on the substrata in T1 would result in higher APA values when concentrations are normalized to chlorophyll *a*. The third possibility is that bacterial APA may have increased independent of any change in heterotrophic biomass. Bacteria biomass was not quantified, so we cannot distinguish between these alternative explanations.

Conclusions

The interactions between autotrophic and heterotrophic processes and the relative dominance of each in providing a food supply to the invertebrate community are important in the context of choice of materials that can be added to nutrient-deficient streams for salmonid enhancement purposes. Grain has been advocated as a desirable supplement (Mundie et al. 1983) because it is inexpensive and provides a direct source of food for a variety of fish food organisms. This proposal was based on experiments conducted in a nutrient-replete side channel of the Big Qualicum River, British Columbia.

As part of the present study, P. A. Slaney (unpubl. data) found that grain additions significantly increased mean weights of coho fry by up to 58% over fry weights in the control reach. By comparison, juvenile coho growth increased rapidly with the onset of fertilizer additions, resulting in up to an 82% gain in mean weights in the same growing season. Mean weights of juvenile steelhead trout were not significantly different between the control and T1 but were 67% heavier in fertilized reaches compared with the control. Hence, while grain additions can increase insect standing crop in nutrient-replete streams (Mundie et al. 1983), increased autotrophic production by nutrient addition results in greater benefit for growth of salmonids in a nutrient-deficient stream.

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NOTES

Bioaccumulation and Metabolism of Tri- and Dialkyllead Compounds by a Freshwater Alga

P. T. S. Wong

Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

Y. K. Chau

Department of Environment, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

and J. L. Yaromich and O. Kramar

Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

Wong, P. T. S., Y. K. Chau, J. L. Yaromich, and O. Kramar. 1987. Bioaccumulation and metabolism of tri- and dialkyllead compounds by a freshwater alga. Can. J. Fish. Aquat. Sci. 44: 1257–1260.

A freshwater green alga, *Ankistrodesmus falcatus*, exposed to solutions of trialkyllead, dialkyllead, and inorganic lead (II) compounds (1 mg/L) for 24 h accumulated these compounds with concentration factors of about 100, 2000, and 20 000, respectively. Incubation of this alga with trimethyllead and dimethyllead species over a long period (28 d) revealed the ability of the organism to metabolize these compounds. The metabolic processes for trimethyllead followed a dealkylation sequence with the formation of dimethyllead and lead (II) compounds. The alga not ony accumulated dimethyllead, but also contained significant amounts of trimethyllead and lead (II) compounds. Neither chemical disproportionation reactions nor photodecomposition could account for the quantities produced.

Une algue verte d'eau douce, *Ankistrodesmus falcatus*, a été exposée à des solutions de plomb-trialkyle, de plomb-dialkyle et de plomb inorganique (II) de concentrations de 1 mg/L. Après 24 h, les facteurs de concentration dans les algues s'élevaient à 100, 2 000 et 20 000, respectivement, pour chacun des composés. La culture prolongée (28 j) de ces algues en présence de plomb-triméthyle et de plomb-diméthyle a montre que ces organismes métabolisaient ces substances. Les processus du métabolisme du plomb-triméthyle comportaient une désalkylation suivie de la formation de plomb-diméthyle et de plomb (II). L'algue ne se limitait pas à accumuler du plomb-diméthyle, mais contenait aussi des quantités appréciables de plomb-triméthyle et de plomb (II). Ni les réactions de dismutation ni la photodécomposition ne pouvaient expliquer les quantités ainsi produites.

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Two types of organolead compounds in the environment have caused much concern because of their inherent toxicity and widespread occurrence (Grandjean and Nielsen 1979; Chau and Wong 1984). One type is tetraalkyllead (R_4Pb) which is volatile and insoluble in water. This includes tetraethyllead (Et_4Pb) and tetramethyllead (Me_4Pb) which are used as antiknock additives in gasoline. These compounds may be degraded in the aquatic environment into trialkyllead (R_3Pb^+), dialkyllead (R_2Pb^{2+}), and finally to inorganic lead (Pb^{2+}) species. There are no known monoalkyllead (RPb^{3+}) compounds due to their extreme instability. Tetraalkyllead compounds have been detected in water, fish, algae, and other organisms from freshwater and marine environments (Sirota and Uthe 1977; Chau et al. 1985). The second type consists of hydrophilic ionic species such as R_3Pb^+ and R_2Pb^{2+} which are used as biocides. Their occurrence in the aquatic environment has also been reported (Chau and Wong 1984; Chau et al. 1985).

Accumulation of R_4Pb has been studied in fish (Maddock and Taylor 1980; Wong et al. 1981) and in algae (Silverberg et al. 1977), but little information is available on the accumulation of R_3Pb^+ and R_2Pb^{2+} by aquatic organisms. In this report, the ability of a freshwater green alga, *Ankistrodesmus falcatus*, to metabolize dimethyllead (Me_2Pb^{2+}) and trimethyllead (Me_3Pb^+) compounds is presented.

Materials and Methods

Trimethyllead acetate and triethyllead acetate (Et_3Pb^+) were obtained from Alfa chemicals (Danvers, MA). Dimethyllead chloride and diethyllead chloride (Et_2Pb^{2+}) were gifts from

Associated Octel Co. (Ellesmere Port, S. Wirral, Great Britain). Tetramethylammonium hydrooxide (TMAH) was from Fisher Chemicals (Toronto, Ont.), sodium diethyldithiocarbamate (NADDTC) from Baker Chemicals (Canlab, Toronto, Ont.) and *n*-butyl Grignard reagent in tetrahydrofuran from Alfa Co. All other reagents and solvents were commercially available in high-purity grade.

An axenic culture of *Ankistrodesmus falcatus* var. *acicularis* (Ontario Ministry of the Environment, Rexdale, Ont.) was grown in 1 L of Bold medium (Nichols and Bold 1965) at 20°C on a rotary shaker (100 rpm) under conditions of 18 h of light (5000 lx) and 6 h of darkness. When the culture reached the logarithmic phase of growth in about 7 d, it was used as inoculum.

Accumulation of R_3Pb^+, R_2Pb^{2+}, and Pb^{2+} by the algae in 24 h — The algal culture was inoculated into six 250-mL Erlenmeyer flasks, each containing 200 mL of Bold medium to give a cell biomass of 7×10^5 cells/mL (62 μg dry wt/mL). Concentrated stock solutions of $Pb(NO_3)_2$, Et_3Pb^+, Me_3Pb^+, Et_2Pb^{2+}, and Me_2Pb^{2+} were prepared in double-distilled water and added to the flasks to give initial concentrations of 1 mg/L as Pb in the medium. The sixth flask without Pb addition was used to determine the Pb contamination from the culture and medium. The flasks were incubated in a shaker under a constant light intensity of 5000 lx. After 24 h of incubation, the cultures were separated from the medium by centrifugation at 16 000 × *g* for 20 min. The pellets were resuspended in 1 mL of the fresh medium. The cell suspensions and 100 mL of the supernates were used for Pb analysis.

Accumulation and degradation of Me_3Pb^+ and Me_2Pb^{2+} by algae in 28 d — Four 10-L glass bottles, each containing 5 L of the medium, were sterilized by autoclaving. Upon cooling, two bottles were inoculated aseptically with 40 mL of the algal culture to give 1.2×10^5 cells/mL (11 μg dry wt/mL). The other two bottles were not inoculated with the culture and were used to determine the process of chemical degradation and photodegradation. A concentrated stock solution of Me_3Pb^+ or Me_2Pb^{2+} was added to each of the two bottles (with and without the culture) to give an initial concentration of 1 mg/L in the medium. Both culture and medium were mixed continuously with a magnetic stirrer and incubated under a constant light intensity of 5000 lx at 20°C. One litre of the medium was removed from each of the four bottles immediately after the addition of the alkyllead compounds and after 7, 14, 21, and 28 d of incubation.

Analyses of alkyllead and Pb species — One hundred millilitres of medium was extracted with 5 mL of aqueous 0.5 *M* NaDDTC, 5 g of NaCl, and 5 mL of benzene for 30 min (Chau et al. 1985). The benzene phase was carefully evaporated in a rotary evaporator to 1 mL in a 15-mL centrifuge tube to which 0.2 mL of *n*-butyl Grignard reagent was added. The mixture was gently mixed for 1 min and washed with 2 mL of 0.5 *M* H_2SO_4. The organic phase was dried in anhydrous Na_2SO_4. Appropriate amounts (10–20 μL) were injected into the gas chromatography – atomic absorption spectrometry (GC-AAS) system for analyses (Chau et al. 1983). Detection limit for Pb in the medium was 8 ng/L.

The algal culture was digested with 5 mL of 20% TMAH in a water bath at 60°C for 2 h. After cooling, the mixture was neutralized with 50% HCl to pH 6–8 and extracted with 3 mL of 0.5 *M* NaDDTC, 2 g of NaCl, and 3 mL of benzene for 1 h.

TABLE 1. Recovery of alkyllead and lead (II) compounds from medium and algae.

Compound	% of recovery	
	Medium	Algae
Pb^{2+}	91	95
Me_2Pb^{2+}	100	90
Me_3Pb^+	100	100
Et_2Pb^{2+}	90	95
Et_3Pb^+	92	100

TABLE 2. Accumulation of alkyllead and lead (II) compounds by *A. falcatus* after incubating in 1 mg/L of the lead compounds for 24 h.

Compound	Concn. of lead compounds		Concn. factors[a]
	Supernate (mg/L)	Cells (μg/g)	
Pb^{2+}	0.31	6242	20 135
Me_2Pb^{2+}	0.71	1978	2 786
Me_3Pb^+	1.02	100	98
Et_2Pb^{2+}	0.94	1821	1 937
Et_3Pb^+	1.00	170	170

[a]Concentration factor = concn. in algae/concn. in supernate.

The mixture was centrifuged and 1 mL of the benzene phase was transferred to a glass-stoppered vial for butylation with 0.2 mL of *n*-butyl Grignard reagent. The mixture was washed with 2 mL of 0.5 *M* H_2SO_4 and the benzene phase was dried in anhydrous Na_2SO_4. Aliquots (10–20 μL) were injected into the GC-AAS system for Pb determination. Detection limit for Pb in algae was 8 ng/g.

Algal cells were enumerated under a microscope with a Petroff–Hausser counting chamber (Frobisher 1968) and the cell number was then related to a previously constructed dry weight curve. All cell count data were based on the average of three separate counts of each sample.

Results and Discussion

Recovery of ionic alkyllead and Pb species from medium and alga — To determine the quantitative recovery of the alkyllead and inorganic Pb compounds from medium and alga, known concentrations of these compounds were added into the medium and algal pellet, incubated for 1 h, and then extracted with benzene (see Materials and Methods). With this technique, there was almost complete recovery (90–100%) of the Pb compounds from the medium and alga (Table 1). Birnie and Hodges (1981) used a combination of solvent extraction and a differential pulse anodic stripping voltammetric technique for the isolation and determination of ionic alkyllead species in tissues of marine fauna. Their recovery of Et_2, Et_3, and Me_3Pb^+ from the tissues was only 60–90%, while the recovery of Me_2Pb^{2+} was poor (10–40%). Since the technique for speciation of ionic alkyllead was not available until recently (Birnie and Hodges 1981; Chau et al. 1983), more data on ionic alkyllead in the aquatic system should be available in the near future.

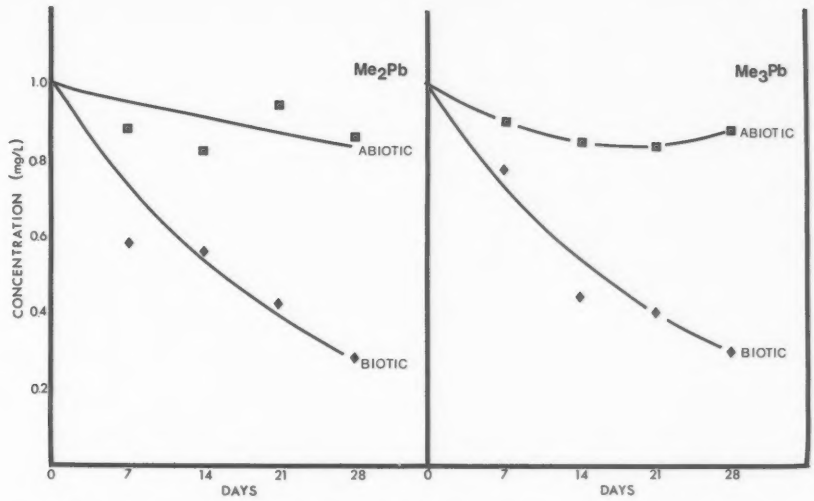

FIG. 1. Rate of biotic and abiotic degradation of Me_2Pb^{2+} and Me_3Pb^+ compounds in 28 d. The data points are means of three samples with standard deviations of less than 18%.

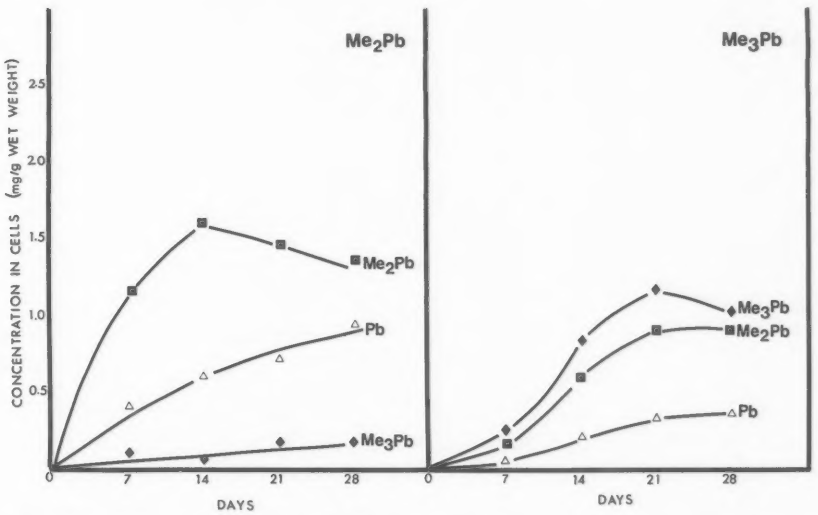

FIG. 2. Accumulation and degradation of Me_2Pb^{2+} and Me_3Pb^+ compounds in *A. falcatus* in 28 d. The data points are means of three samples with standard deviations of less than 10%.

Accumulation of alkyllead and Pb species — *Ankistrodesmus falcatus* was exposed to various alkyllead compounds at 1 mg/L, a level below toxicity (Wong et al., unpubl. data), for 24 h. The short incubation time was used to lessen the possible biotic and/or abiotic degradation of the compounds. The results (Table 2) indicate that the alga was able to accumulate inorganic Pb the most with concentration factor of 20 135, followed by R_2Pb^{2+} (with concentration factors between 1937 and 2786) and R_3Pb^+ (98 and 170). There was no significant relationship between the length of the alkyl chain and accumulation when R was either a methyl or ethyl group. The abiotic degradation of the ionic alkyllead species was negligible, less than 5% in 24 h. Accumulation of inorganic Pb by algae may be by surface adsorption or by absorption into specific cellular components (Schulz-Baldes and Lewin 1976; Wong et al. 1978). The value of 6242 μg inorganic Pb/g wet wt of cells from this experiment was considerably less than 11 640 μg/g in two marine phytoplankton (Schulz-Baldes and Lewin 1976). The differences may be due to the variation in algal species and culture medium used in the experiments. Data on the accumulation of ionic alkyllead compounds by algae are not available. In other organisms, Maddock and Taylor (1980) observed very high concentration factors of R_4Pb in shrimp, mussels, and plaice and about one or two orders of magnitude lower concentration factors for the corresponding R_3Pb^+ derivatives. R_4Pb has been shown to penetrate

the cell membrane and deposited in the cytoplasm of algae and animals (Silverberg et al. 1977; Grandjean and Nielsen 1979).

Uptake and degradation of dimethyllead and trimethyllead — In the experiment on the accumulation of alkyllead and inorganic Pb compounds by the alga (Table 2), the supernatant contained other alkyllead compounds at levels higher than could be explained by abiotic degradation. Since no information is available on the degradation of ionic alkyllead compounds by algae, a study was carried out to determine the possibility of degradation of Me_2Pb^{2+} and Me_3Pb^+ compounds by *A. falcatus*. The alga was incubated with 1 mg/L of the lead compounds for periods up to 4 wk. At various times, the concentrations of the lead compounds in the cells and in the medium were analysed. An identical set without the algal inoculum was also analysed for abiotic degradation. Me_2Pb^{2+} and Me_3Pb^+ in the medium alone (no algae) were quite stable (less than 20% decomposition) under light over 4 wk of incubation (Fig. 1). The decomposition of Me_2Pb^{2+} yielded Me_3Pb^+ and Pb^{2+}, while Me_3Pb^+ yielded Me_2Pb^{2+} and Pb^{2+}. Chau and Wong (1984) also reported that R_2Pb^{2+} may be chemically rearranged to R_3Pb^+ and Pb^{2+}. Similary, Jarvie et al. (1981) observed that Me_2Pb^{2+} and Me_3Pb^+ were decomposed very slowly in the presence of light with 4–10% loss in 30 d of incubation.

In the presence of *A. falcatus*, the disappearance of the alkyllead compounds was much more rapid (Fig. 1). An estimated loss of 50% occurred in about 15 d. The disappearance of the alkyllead compounds from the medium mirrored their appearance in the cells (Fig. 2). Me_2Pb^{2+} was taken up quite rapidly and accumulated to a concentration factor of 1.6×10^3. There was a lag period in the uptake of Me_3Pb^+ and the concentration factor reached only 1.1×10^3. The initial rates of uptake of Me_2Pb^{2+} and Me_3Pb^+ were estimated as 0.2 and 0.06 mg·g wet wt cells^{-1}·d^{-1}, respectively. The same algal species accumulated tributyltin species by a factor of 8×10^3 (Maguire et al. 1984). Concurrent with the accumulation of Me_2Pb, the alga also contained significant amounts of Me_3Pb^+ and Pb^{2+} (Fig. 2). Similarly, Me_2Pb^{2+} and Pb^{2+} were detected in the cells incubated with Me_3Pb^+. It seemed unlikely that the products in the cells could derive solely from the disproportionation of Me_3Pb^+ and Me_2Pb^{2+} in the medium, since the levels of these compounds were much higher than could be explained by the slow disproportionation. Dealkylation of R_3Pb^+ and R_2Pb^{2+} in animals have been reported (Jensen 1984). The mechanism for the dealkylation is believed to be through lipid peroxidation. Studies by Roderer (1984) suggested that algae could not metabolize R_4Pb to R_3Pb^+. However, no study was available until now to indicate that algae could metabolize Me_3Pb^+ and Me_2Pb^{2+}. The results also suggest that algae can play an important role in the cycling of lead compounds in aquatic ecosystems.

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Acoustical Assessment of Squid (*Loligo opalescens*) off the Central Oregon Coast

Katharine Jefferts

College of Oceanography, Oregon State University, Corvallis, OR 97331, USA

Janusz Burczynski

BioSonics, Inc., 4520 Union Bay Place NE, Seattle, WA 98105, USA

and William G. Pearcy

College of Oceanography, Oregon State University, Corvallis, OR 97331, USA

Jefferts, K., J. Burczynski, and W. G. Pearcy. 1987. Acoustical assessment of squid (*Loligo opalescens*) off the central Oregon coast. Can. J. Fish. Aquat. Sci. 44: 1261–1267.

Squid (*Loligo opalescens*) were surveyed acoustically in an area off the Oregon coast using dual-beam and echo integrator techniques. The method developed is shown to be feasible for in situ estimation of target strength, distribution, and abundance of midwater squids. Average target strength was estimated both while drifting with a squid school and cruising over one. The values derived, −58.6 to −58.7 dB, are much lower than previous estimates, but more realistic in relation to the target strength of fishes. Schools judged to be fish or squid showed little difference in depth or size, but varied significantly in geographic distribution. Density estimates ranged from 9.9 t/km^2 in a known spawning area to 0.93 t/km^2 in an adjacent area.

Une relevé acoustique du calmar (*Loligo opalescens*) a été effectué dans les eaux au large de la côte de l'Oregon à l'aide de dispositifs d'intégration échographique et de faisceaux jumelés. Les auteurs montrent que la méthode mise au point pour l'estimation *in situ* de l'index de réflexion de la cible, de la répartition et de l'abondance du calmar mésopélagique est réalisable. On a déterminé l'index moyen de réflexion de la cible en dérivant avec un banc de calmar et en passant lentement au-dessus d'un banc. Les valeurs ainsi obtenues, soit de −58,6 à −58,7 dB, sont très inférieures aux estimations précédentes quoique plus réalistes par rapport à l'index de réflexion des poissons. Les bancs que l'on a déterminé être des poissons ou des calmars montraient peu de différence pour la profondeur ou la taille mais variaient fortement pour ce qui est de la répartition géographique. Les estimations de la densité varient de 9,9 t/km^2 dans une frayère connue à 0,93 t/km^2 dans une région avoisinante.

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The squid *Loligo opalescens* occurs from Baja California to southeastern Alaska (Fields 1965; Okutani and McGowan 1969; Street 1982). It has been fished historically off California (Kato and Hardwick 1975); a fishery has recently developed off Oregon, and there is some potential for expansion in the northerly portions of its range (Bernard 1980; Street 1982). Throughout its range, little is known about the distribution, abundance, or availability of its stocks. At the minimum, some estimate of stock size is needed to manage this resource.

This study was initiated to evaluate the usefulness of acoustical techniques for estimating target strength, population density within a localized area, and the size of squid schools. Acoustic methods were applied that have been successfully used for fish stocks, specifically a dual-beam technique to estimate target strength, and echo integration to obtain quantitative information on numbers and biomass (Nakken and Venema 1984).

Previous acoustic work with squids has been limited. Echogram patterns have been described for *Todarodes pacificus* (Shibata and Flores 1972) and *Loligo opalescens* (Vaughan and Recksiek 1978, 1979). Kawaguchi and Nazumi (1972) described optimum recording conditions for *Todarodes*: narrow beam, minimum pulse-length echosounders operating at 75–200 kHz.

Methods

The acoustic survey was carried out on 21 and 23–25 May 1984 off the central Oregon coast in an area extending from about 56 km south of Newport, Oregon, to about 74 km north of Newport (Fig. 1; Table 1). Survey transects were laid out in a zigzag pattern between the 27- and 55-m isobaths, the depths where commercial fishing for squid is most common in this area. Echo integrator data were collected along survey transects to determine vertical and horizontal distribution of squid schools. Two paper records were made during the survey: a wet paper record made with a Japan Radio Company 999 Zoom sounder (JRC) at 50 kHz and a dry paper record at 120 kHz made with a Ross Scientific Recorder. To facilitate target identification, a Furuno FCV 201 28 kHz color sounder (beam width 30 × 34°) was run simultaneously. Echo groups

TABLE 1. Summary of survey data.

Date (May 1984)	Time of survey (PDT)	Max. and min. latitude (°N) of survey	Time of echo integrator survey
21	15:23–20:34	44°34.90′ to 44°11.39′	19:01–20:54
23	14:46–21:13	44°36.10′ to 44°8.94′	14:59–21:33
24	08:45–14:56	45°15.79′ to 44°37.26′	08:52–14:56
25	09:47–10:31	44°16.53′ to 44°8.90′	JRC record only

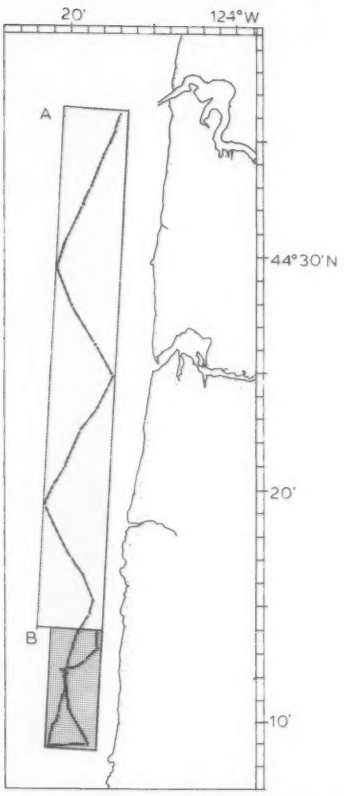

FIG. 1. Survey transect for 23 May.

or schools were classified as fish or squid by the skipper, who was experienced in fishing for squid and interpreting sounder outputs. Classification was based on the shape, intensity (color), and depth of echo groups. Target identification was confirmed on several occasions by jigging and purse seining.

Acoustic data were obtained from four days of cruises (Table 1). On 21 May the area from Newport south to near Tenmile Creek was surveyed (Fig. 1). Echo integrator and target strength data were obtained and 6.3–7.3 t of *Loligo* was landed (average size 11.6 cm dorsal mantle length (DML), 23.7 g). The cruise on 22 May was curtailed due to foul weather. The area south of Newport to Heceta Head was also surveyed on 23 May; target strength measurements were again made. On 24 May the area from Newport north nearly to Cape Lookout was surveyed; only echo integrator data were obtained. On 25 May a short echo integrator survey was made north from Heceta Head; dual-beam data were collected. Two purse seine sets early on 25 May produced a total of about 7.7 t of squid.

The echo integrator data for 23 May were the only ones processed. The data from 23 May were technically the best and represented a large geographic area containing large quantities of squid. We felt that processing the echo integrator data from the other dates was not justified, for the following reasons: 21 May, the acoustic system parameters were repeatedly changed to optimize system performance; 24 May, very few squid were encountered; 25 May, the survey was abbreviated because of weather conditions and the chart recorder and navigation system failed. The JRC wet paper records from the other dates were analyzed, however, to ascertain the distribution of fish and squid schools.

A commercial purse seine (365 m long × 38 m deep) was used to sample squid schools in the study area, usually after dark. Lights (40 kW) were used to attract squid around the vessel. Dual-beam data for target strength estimation were obtained from visually verified squid schools prior to purse seining. Samples of squid from purse seining were retained for measurements of DML and weight.

Hydroacoustic system — The hydroacoustic data collection system consisted of a 120-kHz dual-beam transducer (nominal beam width 10°/22°) mounted on a BioSonics towed body, a BioSonics model 101 dual-beam echo sounder, a BioSonics model 121 echo integrator, a modified Ross 250 E chart recorder, an oscilloscope, digital cassette recorder, and an Otrona Attache computer.

The transducer was towed about 1 m below the surface at about 4 m/s. Pulse rate was 4 pings/s, and echo integrator processing interval (one sequence) was 250 pings, for a sampling distance unit of 250 m.

The echo integrator data were collected from twenty-nine 2-m depth intervals with the narrow beam (10°) transducer and 20 log R time-varied gain (TVG). The echo integrator output data were transferred in real time to the computer and stored on disc.

The dual-beam data were digitally recorded on a magnetic video cassette via a Sony digitizer system, using 40 log R TVG on both the narrow and wide channels of the echo sounder.

Data analysis — The dual-beam data were processed ashore (at BioSonics) using a BioSonics model 181 dual-beam processor system. The processor selected echoes from individual squid (using a pulse width criterion at two pulse amplitudes), extracted the target strength from the echo level out of the sounder, and calculated various statistics for the individual squid target strength (Ehrenberg 1984). The dual-beam system calibration was verified in the field using a ping-pong ball as the standard target. The measured target strength was −41.9 dB, which is reasonable for an air-filled sphere the size of a ping-pong ball (theoretical value −40.5 dB).

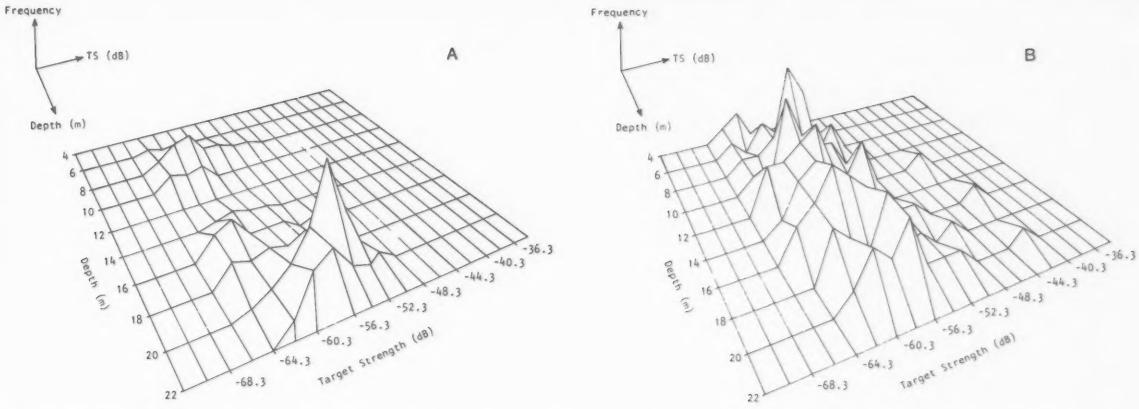

FIG. 2. Target strength distribution of individual squids sampled by the dual-beam system with (A) vessel cruising (25 May, 00:30, latitude 45°N) and (B) vessel drifting (25 May, 00:00, latitude 45°N).

The raw survey data from the echo integrator were edited by setting the integrator output values to zero for all targets not identified as squid. The integrator output was scaled to yield absolute squid density (number of squid per cubic metre) by applying a scaling factor based on system parameters and target strength results from the dual-beam data. The scale factor accounts for the transmitting and receiving response of the acoustic system, the beam pattern of the transducer, and the average target strength of individual squid in the population. All terms in the scale factor except the average individual squid target strength were determined from the acoustic system calibration data. The average target strength was determined from the in situ dual-beam data.

The total number of squid in the surveyed region was estimated by multiplying the calculated squid densities by the total volume of water in the regions surveyed. The area of the survey was estimated planimetrically.

Biomass was estimated by multiplying the total number of squid by the average weight of squid sampled from the catch. Confidence limits were obtained by assuming (for simplicity) that the estimates obtained from the 1-min integrator sequences were independent, identically distributed random variables. We felt that 1-min sequences were sufficiently short to allow such an assumption.

The traces on the JRC paper records were classified as squid schools, fish schools, or single unidentified targets.[1] As a rough estimate, a school's size was estimated by measuring its width on the echogram (length of time insonified) and multiplying by vessel speed. The small size of many schools and expansion of the acoustic beam with depth prevent very accurate school size estimates; the data were depth stratified to ameliorate the beam expansion problem. Single targets were not counted in 0- to 9-m interval due to a low signal to noise ratio; however, distinct schools were visible.

Results

Target strength — The target strength measurements were all made on a large spawning concentration of *Loligo opalescens* north of Heceta Head. Dual-beam data were recorded in two modes when squid were aggregated near the vessel at night: drifting and underway over the aggregation. The average target strength values were as follows: drifting mode, −58.6 dB (SD 4.1 dB); cruising mode, −58.7 dB (SD 3.2 dB). The frequency distributions of target strengths of echoes at different depths are different for cruising and drifting modes (Fig. 2), probably due to avoidance of the moving vessel by squid. Target strength was not apparently affected by such avoidance reactions.

The average target strength value for individual squid is much lower than for fish of similar size. This was expected due to the lack of a gas-filled swimbladder in squid. The target strength for several species of fish (with swimbladders) of the same size[2] as the squid taken in this study (mean 11.6 cm DML, 23.7 g) is about −43 dB at a frequency of 120 kHz (Love 1971). The value obtained for squid is about 15 dB lower.

Biomass estimates — The geographic area surveyed on 23 May was divided into two areas because of a significant difference in density, both of squid schools and of echo integrator data points. The echo integrator output (Tables 2 and 3) is codified as number of squid in the area: area A, 7.856×10^6 (SD 3.242×10^6); area B, 1.437×10^7 (SD 3.998×10^6). Biomass was estimated at 186 t for area A and 341 t for area B using an average squid weight of 23.7 g. Average densities were 0.927 t/km^2 in area A and 9.91 t/km^2 in area B, or roughly 10 times higher than in area A.

School characteristics — Squid schools were much more abundant in the southern area: 1.45–2.20 schools were encountered per kilometre (May 21, 23, 25, Table 4) compared with 0.34 in the area north of Newport (May 24, Table 4). Fish school and single target occurrences showed no such trend, ranging from 0.50–1.01 and 1.20–48.2 encounters/km in the southern area to 1.14 and 27.7 in the northern area, respectively.

[1]Single targets were defined as targets so small that they only marked on one pass of the recording pen; their identification is problematic.

[2]Total length of fish was related to DML of squid. The difference in target strength would be even greater were total length of fish related to total length of squid.

TABLE 2. Integrator output for area A. Surface area of region = 2.0055×10^8 m^2.

Depth stratum (m)	Estimated density (squid/m^3)	Total number of squid	Confidence limits (95%)
0–2	0.0000	0.00000	0.0000
2–4	0.4312×10^{-3}	0.17294×10^6	0.1669×10^6
4–6	0.7087×10^{-3}	0.28427×10^6	0.4094×10^6
6–8	0.6262×10^{-3}	0.25115×10^6	0.3519×10^6
8–10	0.1435×10^{-2}	0.57540×10^6	0.7582×10^6
10–12	0.5308×10^{-3}	0.21289×10^6	0.2432×10^6
12–14	0.2358×10^{-3}	0.94579×10^5	0.1439×10^6
14–16	0.3715×10^{-4}	0.14900×10^5	0.2345×10^5
16–18	0.2373×10^{-4}	0.95189×10^4	0.1248×10^5
18–20	0.2228×10^{-3}	0.89349×10^5	0.1842×10^7
20–22	0.5881×10^{-4}	0.23588×10^5	0.2536×10^5
22–24	0.8253×10^{-5}	0.33102×10^4	0.3231×10^4
24–26	0.8466×10^{-4}	0.33928×10^5	0.3554×10^5
26–28	0.8454×10^{-3}	0.33497×10^6	0.3350×10^6
28–30	0.7895×10^{-3}	0.30613×10^6	0.2163×10^6
30–32	0.1827×10^{-2}	0.69309×10^6	0.4659×10^6
32–34	0.5058×10^{-2}	0.18514×10^7	0.2410×10^7
34–36	0.4931×10^{-2}	0.16478×10^7	0.1530×10^7
36–38	0.2538×10^{-2}	0.71580×10^6	0.5334×10^6
38–40	0.1050×10^{-2}	0.23735×10^6	0.3054×10^6
40–42	0.1529×10^{-2}	0.29001×10^6	0.7829×10^6
42–44	0.2154×10^{-4}	0.28789×10^4	0.1138×10^5
44–46	0.1514×10^{-3}	0.11150×10^5	0.5721×10^5
46–48	0.0000	0.00000	0.0000
48–50	0.0000	0.00000	0.0000
50–52	0.0000	0.00000	0.0000
52–54	0.0000	0.00000	0.0000
54–56	0.0000	0.00000	0.0000
56–58	0.0000	0.00000	0.0000
Total		0.78564×10^7	0.3242×10^7

TABLE 3. Integrator output for area B. Surface area of region = 3.637×10^7 m^2.

Depth stratum (m)	Estimated density (squid/m^3)	Total number of squid	Confidence limits (95%)
0–2	0.0000	0.00000	0.0000
2–4	0.2330×10^{-1}	0.16949×10^7	0.1670×10^7
4–6	0.2821×10^{-1}	0.20518×10^7	0.1616×10^7
6–8	0.7144×10^{-2}	0.51962×10^6	0.7681×10^6
8–10	0.3336×10^{-2}	0.24268×10^6	0.2582×10^6
10–12	0.5024×10^{-2}	0.36547×10^6	0.3602×10^6
12–14	0.1734×10^{-1}	0.12611×10^7	0.1045×10^7
14–16	0.2451×10^{-1}	0.17830×10^7	0.1333×10^7
16–18	0.2116×10^{-1}	0.15392×10^7	0.9517×10^6
18–20	0.1857×10^{-1}	0.13500×10^7	0.1842×10^7
20–22	0.9258×10^{-2}	0.66217×10^6	0.7504×10^6
22–24	0.7353×10^{-2}	0.48518×10^6	0.6906×10^6
24–26	0.4392×10^{-2}	0.24504×10^6	0.2420×10^6
26–28	0.5175×10^{-2}	0.25314×10^6	0.3874×10^6
28–30	0.3951×10^{-3}	0.18383×10^5	0.2701×10^5
30–32	0.2534×10^{-2}	0.11435×10^6	0.2008×10^6
32–34	0.1382×10^{-1}	0.60137×10^6	0.7736×10^6
34–36	0.8123×10^{-2}	0.34070×10^6	0.5148×10^6
36–38	0.5888×10^{-2}	0.23218×10^6	0.2863×10^6
38–40	0.5308×10^{-2}	0.19281×10^6	0.2844×10^6
40–42	0.3764×10^{-2}	0.12180×10^6	0.1686×10^6
42–44	0.8765×10^{-2}	0.23192×10^6	0.4724×10^6
44–46	0.4423×10^{-2}	0.70527×10^5	0.2506×10^6
46–48	0.9215×10^{-5}	0.78233×10^2	0.5171×10^3
48–50	0.0000	0.00000	0.0000
50–52	0.0000	0.00000	0.0000
52–54	0.0000	0.00000	0.0000
54–56	0.0000	0.00000	0.0000
56–58	0.0000	0.00000	0.0000
Total		0.14377×10^8	0.3998×10^7

The ratio of squid schools to total schools (fish plus squid) encountered also showed a major difference between the southern transects and the northern one. The three transects south of Newport found squid schools to be more abundant, comprising 65–77% of the schools, while the northern transect showed fish schools to be relatively more abundant, comprising 77% of encounters.

School size distribution (Fig. 3) showed little variation in mean size of schools judged to be squid or fish during the 4 d. Squid schools were usually 3.3–7.8 or 7.9–15.4 m long, while fish schools were always in the latter length interval. Squid school size varied from a mean of 8.74 m on 25 May to 22.9 m on 24 May, while the mean size of fish schools varied from 10.1 to 14.6 m. The variance was much higher for squid than fish schools, due to the presence of a few very large squid schools (>200 m) (Table 4).

Depth distribution of the schools was variable (Fig. 4). Maximum abundance of both squid and fish schools occurred on three of four dates between 9 and 18 m. Single targets were most abundant twice in the 18- to 27-m interval and once each in the 9- to 18-m and 27- to 37-m intervals.

Few data on day–night differences are available.

Discussion

We have shown that hydroacoustic systems can be used to provide reasonable target strength and biomass measurements for spawning aggregations of squid. The acoustic assessment techniques developed to date require that the acoustic returns from the species of interest can be identified, which can be difficult in a mixed species population. However, several characteristics of the *Loligo opalescens* spawning aggregation help to alleviate this difficulty. This squid spawns in relatively shallow water close to shore; thus, target identification may be possible visually from the surface, aerially, with underwater video equipment, or physically (by capture with nets or jigs). In the limited geographic area investigated, squid fishermen appear to be able to identify squid schools based on characteristics of a chromoscope echogram, by experience with a particular school, and by nightlighting. Flocks of feeding birds, especially the common murre (*Uria aalge*), are also used as indicators of squid schools. The squid appear to occur in discrete schools (bycatch was minimal, amounting to very few groundfish or salmonids per set — several tons of squid), although the schools are small outside the spawning area. Data on spawning condition in the schools seem to back up the fishermen's belief that sequential batches of squid spawn in the same local area (S. Schones and P. Schones, Box 17, Old River Rd., Siletz, OR, pers. comm.; Starr and McCrae 1984). In the area studied here, Starr and McCrae (1984) observed a decrease in the ratio of mantle weight to mantle length, the result of spawning activity. This was followed by an increase in the weight to length ratio near the end of harvesting operations, indicating an influx of animals at an earlier stage of

TABLE 4. Characteristics of fish and squid schools and unidentified single targets. Day, date (May 1984); Min, minutes of survey time; Nbr, number of schools or single targets; Freq, average number of schools or targets per minute; *L*, average horizontal dimension of schools (metres); SD, standard deviation associated with average size.

		Squid				Fish				Single targets	
Day	Min	Nbr	Freq	L	SD	Nbr	Freq	L	SD	Nbr	Freq
21	311	120	0.39	12.7	15.7	55	0.18	10.1	5.57	2628	8.5
23	407	116	0.29	14.6	26.8	35	0.09	10.1	5.00	85	0.21
24	371	22	0.059	22.9	23.8	73	0.20	14.6	13.3	1778	4.8
25	44	15	0.34	8.74	4.33	8	0.18	12.1	5.88	32	0.73

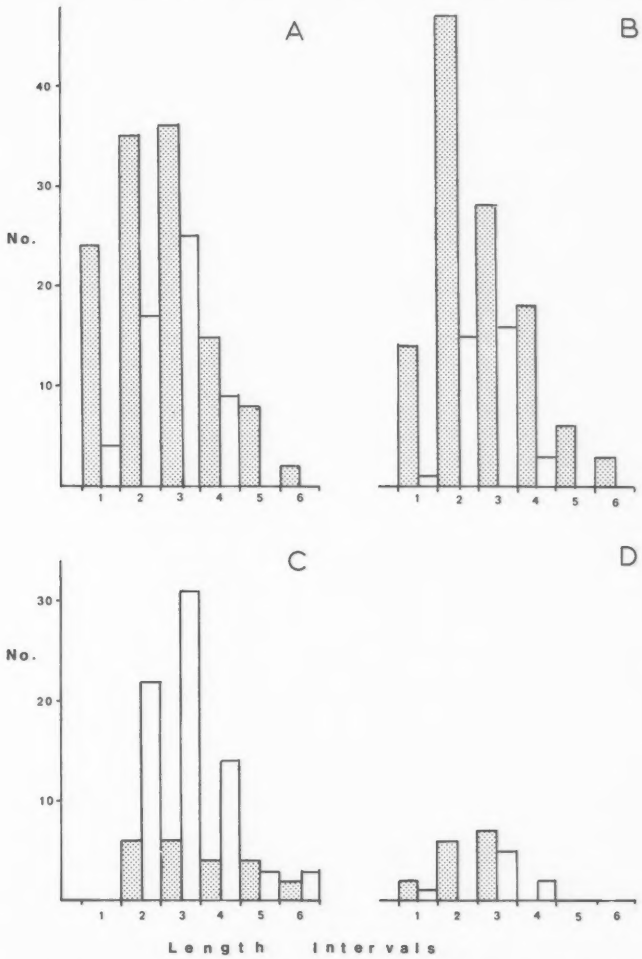

FIG. 3. Length–frenquency distribution of fish and squid schools for (A) 21 May, (B) 23 May, (C) 24 May, and (D) 25 May. Length interval: 1 = 0–3.2 m; 2 = 3.3–7.8 m; 3 = 7.9–15.4 m; 4 = 15.5–30 m; 5 = 31–60 m; 6 = >60 m. Stippled bars represent squid schools; open bars represent fish schools.

maturity. Their data indicate that, in all, three groups of squid spawned sequentially in the area studied here.

Prior to this study, no in situ measurements of the target strength of squid had been reported, although a limited number of measurements on dead, tethered squid had been made. Matsui et al. (1972) estimated a maxuimum target strength of −45 dB at 50 kHz and −42 dB at 200 kHz for *Doryteuthis bleekeri* (11–12 cm DML). Target strengths of about −39 dB at 200 kHz have been measured for *Loligo opalescens* (100–133 mm DML; Vaughan 1978; Cailliet and Vaughan

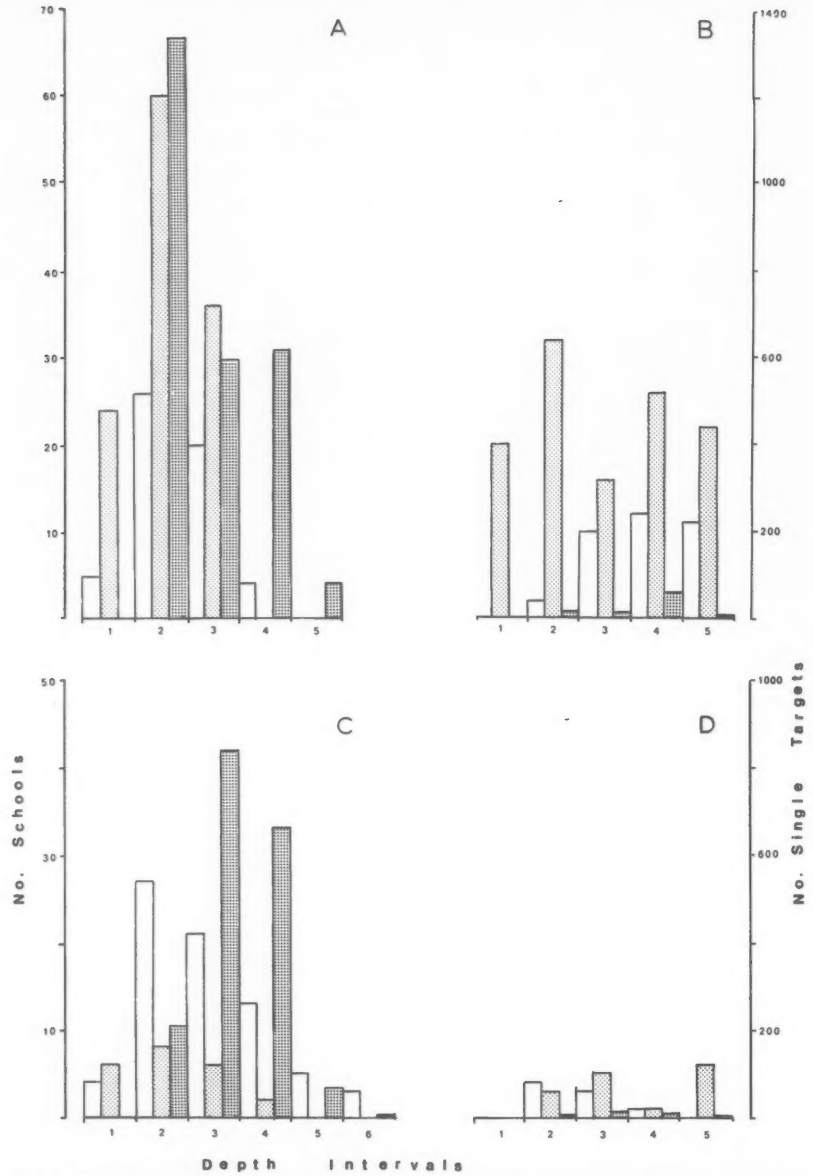

FIG. 4. Depth distribution of fish and squid schools for (A) 21 May, (B) 23 May, (C) 24 May, and (D) 25 May. Depth interval: 1 = 0–9 m; 2 = 9–18 m; 3 = 18–27 m; 4 = 27–37 m; 5 = 37–46 m; 6 = 46–55 m. Open bars represent fish schools; light-stippled bars represent squid schools; dark-stippled bars represent single targets.

1983 recommended a figure of 41.1 dB (*sic*) for squid of the same size) and −47.5 to −37.5 dB for *Loligo formosana* (11–19 cm DML; Shibata and Masthawee 1980). These values seem too large based on target strength values obtained from comparably sized fishes with swimbladders, which should be much better acoustic reflectors.

In addition, the target strength information appears to be independent of avoidance behavior. Essentially identical target strengths were calculated for schools exhibiting and not exhibiting such avoidance.

We have shown that off the central Oregon coast, spawning aggregations of *Loligo opalescens* are highly concentrated. Outside such aggregations, preliminary data suggest that squid form small and relatively infrequent schools. Thus, the concentrations which are being fished probably represent a large fraction of the population which is spawning at that time. Repeated acoustic survey of a relatively small area may thus provide a useful estimate of the vulnerable spawning biomass.

Larger scale surveys are necessary at longer intervals to identify the spawning concentrations.

In summary, we have shown that dual-beam and echo integration techniques are feasible methods for estimating both target strength and biomass in spawning aggregations of squid. This is an important result, as it shows that squid are amenable to acoustic assessment techniques when spawning behavior produces large single-species aggregations.

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